# Autosampler 3950

## with an injection pressure maximum of 700 bar

# User Manual

V1511A

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**Note** For your own safety, **read** the manual and **always** observe the warnings and safety information on the device and in the manual!

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# Intended Use

**Note** Only use the device for applications that fall within the range of the intended use. Otherwise, the protective and safety equipment of the device could fail.

## Device Overview

**Autosampler 3950** The Autosampler 3950 was developed for chromatographic analyses in the high pressure (HPLC) range. An injection valve guarantees precise injection volumes maximum 700 bar. There is an additional variant available offering temperature control. The device features fast sample injection, rapid flushing cycles and high sample throughput. The Autosampler is generally installed as a core element in an HPLC system.

**Legend**

① Opening for capillary feed

② LED status indicator

③ Removable cover

④ Inward sliding glass door

⑤ Temperature control with coolunit inside the Autosampler

⑥ Hose connectors

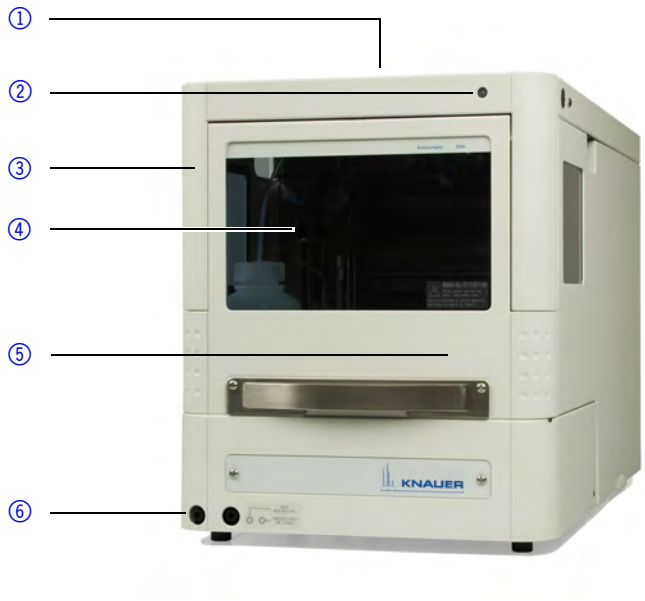



**Fig. 1 Autosampler 3950, with optional temperature control and coolunit**

**Operating Range** Usage is possible in laboratories of universities and research institutions and in industrial and manufacturing routine laboratories in the following areas:

- Biochemistry analyses
- Chemical analyses
- Food analyses
- Pharmaceutical analyses
- Environmental analyses

# Features

## Standard

- Injection valve with injection pressure maximum of 700 bar
- Injection volume for HPLC of maximum 5000 µl
- Temperature control between 4 – 40 °C[1]
- Flexibly equipped with microtiter plate or standard sample plate
- Maximum sample capacity of 768 Wells or 96 standard Autosampler vials
- Injection cycle < 60 s,  60 s incl. cleaning
- Full loop or partial loop injection or microliter pick-up injection
- Quick-exchange injection valve
- Sample priority function
- High-resolution syringe controller

## Optional Components

KNAUER offers the device in two variations:

- Autosampler without temperature control
- Autosampler with temperature control

1) Relevant to Autosampler 3950 with temperature control

# Eluents

Even small quantities of other substances, such as additives, modifiers, or salts can influence the durability of the materials.

**Note** The list of selected solvents was compiled based on research in the pertinent literature and is only a recommendation. If there is any doubt, contact the technical support of the manufacturer.

| Suitable eluents | Less suitable eluents | Not suitable eluents |
|---|---|---|
| ▪ Acetone<br>▪ Acetonitrile<br>▪ Benzene<br>▪ Chloroform<br>▪ Ethyl acetate<br>▪ Ethanol<br>▪ Hexane/heptane<br>▪ Isopropanol<br>▪ Carbon dioxide (liquid 99.999% $CO_2$)<br>▪ Methanol<br>▪ Phosphate buffer solutions (0.5 M)<br>▪ Toluol<br>▪ Dilute ammonia solution<br>▪ Dilute acetic acid (10-50%), at 25° C<br>▪ Dilute sodium hydroxide (1M)<br>▪ Water | ▪ Dimethyl sulfoxide (DMSO)<br>▪ Slightly volatile eluents<br>▪ Methylene chloride<br>▪ Tetrahydrofuran (THF)<br>▪ Dilute phosphoric acid | ▪ Halogenated hydrocarbons, e.g. Freon®<br>▪ Concentrated mineral and organic acids<br>▪ Concentrated bases<br>▪ Eluents containing particles<br>▪ Perfluorinated eluents, e. g. Fluorinert® FC-75, FC-40<br>▪ Perfluorinated polyether, e.g. Fomblin® |

## Flushing Solution

Do not use a salt or buffer solution for flushing!

# Safety for Users

**Professional group**

The user manual is addressed to persons that are qualified as chemical laboratory technicians or have completed comparable vocational training.

The following knowledge is required:

- Basic knowledge of liquid chromatography
- Knowledge regarding substances that are suitable only to a limited extent for use in column liquid chromatography
- Knowledge regarding the health risks of chemicals

If you do not belong to this or a comparable professional group, you may not perform the work described in this user manual under any circumstances.

**What must be taken into account?**

- All safety instructions in the user manual
- The environmental, installation and connection specifications in the user manual
- National and international regulations pertaining to laboratory work
- Original spare parts, tools, and eluents made or recommended by KNAUER
- Good Laboratory Practice (GLP)
- For development of methods and validation of devices: Protocol for the Adoption of Analytical Methods in the Clinical Chemistry Laboratory, American Journal of Medical Technology, 44, 1, pages 30-37 (1978)
- Accident prevention regulations published by the accident insurance companies for laboratory work

More safety-relevant information is listed in alphabetical order in the following table:

| Topic | Explanations |
|---|---|
| Decontamination | Contamination of devices with toxic, infectious or radioactive substances poses a hazard for all persons during operation, repair, sale, and disposal of a device. All contaminated devices must be properly decontaminated. All materials or fluids used for decontamination must be collected separately and disposed of properly. |
| Flammability | Organic eluents are highly flammable. Since capillaries can detach from their screw fittings and allow eluent to escape, it is prohibited to have any open flames near the analytical system. |
| Leaks | Regularly check if any system components are leaking. |
| Solvent tray | Risk of electrical shock or short circuit if liquids get into the device's interior. For this reason, place all bottles in a solvent tray. |

| Topic | Explanations |
|---|---|
| Eluent lines | Install capillaries and hoses in such a way that liquids cannot get into the interior in case of a leak. |
| Power strip | If several devices are connected to one power strip, always consider the maximum power consumption of each device. |
| Power cable | Defective power cables are not to be used to connect the device and the mains power. |
| Self-ignition point | Only use eluents that have a self-ignition point higher than 150 °C under normal ambient conditions. |
| Power supply | Only connect devices to voltage sources, whose voltage equals the device's voltage. |
| Toxicity | Organic solvents are toxic above a certain concentration. Ensure that work areas are always well-ventilated! Wear protective gloves and safety glasses when working on the device! |

**Where is use of the device prohibited?** Never use the system in potentially explosive atmospheres without appropriate protective equipment. For further information, contact the Technical Support of the manufacturer.

**Take the device out of operation** At any time, take the device completely out of operation by either switching off the power switch or by pulling the power plug.

**Opening the Device** The device may only be opened by the technical support of the manufacturer.

# Definition of Personal and Material Damage

Possible dangers related to the device are divided in personal and material damage in this user manual.

| Type of damage | Category | Explanations |
|---|---|---|
| Personal and material damage | DANGER! | Lethal or very serious injuries can occur. |
| | WARNING! | Serious injuries can occur. |
| | CAUTION! | Moderate injuries can occur. Device defects can occur. |

# Symbols and Signs

| | Symbol | Meaning |
|---|---|---|
| **CE mark** | CE | CE (Conformité Européenne) mark for equipment that complies with the pertinent EU directives and comes with a declaration of conformity from the manufacturer. |
| **Warning signs** | | Heavy lifting hazard, danger to the back |
| | | Electrostatic-discharge hazard |
| | | Danger of stab wounds to hands |
| Mandatory signs | | Wear protective gloves. |

# Installation

## Scope of Delivery

**Note** Only use original parts and accessories made by KNAUER or a company authorized by KNAUER.

| | | |
|---|---|---|
| **Delivery** | Autosampler and 500 µl syringe | ☐ |
| | User manual German/English | ☐ |
| | ▪ Cable:<br>- Power Cable<br>- Network cable<br>- I/O interface cable, 9 pins | ☐ |
| | CD: *Autosampler 3950 Service Manager* | ☐ |
| **Accessories kit** | Sample loop, 10 µl and 100 µl | ☐ |
| | PTFE tube, 300 cm in length | ☐ |
| | Silicon drainage tube, 200 cm | ☐ |
| | 2 x vial plate for 48 vials, 1.5 ml | ☐ |
| | 5 x microtiter plate, 96 Wells | ☐ |
| | 2 x 2.5 A fuses | ☐ |
| | 1 x wash bottle | ☐ |
| | Optional: Temperature control | ☐ |

### Checking the Scope of Delivery

1. Check whether the device and accessories are complete.
2. If a part is missing, inform the technical support of KNAUER.

# Unpacking and Setup

## Packaging and Transport

At the factory, the device is carefully packed for safe transport.

**Note** Check for damage caused during transportation. In case you notice any damage, contact the technical support and the forwarder company.

## Contacting the Technical Support

You have various options to contact the Technical Support:

**Phone** +49 30 809727-111

**Fax** +49 30 8015010

**E-mail** support@knauer.net

You can make your requests in English and German.

## Location Requirements

**CAUTION! Defect of the device due to overheating!**
**Set up the device so that it is protected against exposure to direct sunlight.**
**Make sure the room is well-ventilated.**
**Allow 30-cm space at the rear of the device for air circulation.**

**Requirements** The location for the device must meet the following requirements:

- Protect from heavy ventilation
- Weight of the Autosampler: 21 kg (with temperature control)

Dimensions (width × height × depth): 300 × 377 × 577 mm

- Power supply: 100–240 V DC
- Air humidity: < 90 %, non-condensing
- Temperature: 4 – 40 °C
  39,2 – 104 °F

## Space requirements

Side clearance to other devices:

- If there is a device on one side, min. clearance of 5 cm.
- If there are devices on both sides, min. clearance of 10 cm.

## Unpacking

**WARNING! Back injuries while lifting or carrying the device caused by its heavy weight.**
**To avoid injuries, you should ask a second person for help.**

Store all packing material. Retain included packing list carefully for repeat orders.

**Tools** Utility knife

**Procedure**

1. Setup the delivery in such a way that the label is in the correct position. Using the utility knife, cut the adhesive tape. Open the packaging.
2. Remove the foam insert. Take out the accessories kit and the manual.
3. Open the accessories kit and take out all accessories. Check the scope of delivery. In case any parts are missing, contact the technical support.
4. Clasp the device at its side panels and lift it out of the packaging.
5. Remove the foam inserts from the device.
6. Check for damage caused during transportation. In case you notice any damage, contact the technical support.
7. Set-up the device in its location.
8. Remove the adhesive tape from the door of the Autosampler that was used as transportation protection.

# Startup

**Note** Before initial startup, wait approximately one hour until the temperature of the device has adapted to the ambient temperature.

**Auto-Injection System** The speed of the auto-injection system has been increased to fulfill the requirements of ultra-high performance liquid chromatography. However, be aware that the high speed of the auto-injection system can cause stab injuries when handled inappropriately!

## Front View of the Device

The door of the Autosampler can be pushed horizontally into the interior of the device. The front paneling can be completely removed. In order to replace the vial plate, the cooler cover can be removed.

**CAUTION! Personal injuries can occur when the front panel is open or has been removed. Always operate the autosampler with its front panel closed.**

**Legend**

① LED status indicator

**Fig. 2 front view of the device**

**Opening the door**

Position your hands on both sides of the door ① and open. As shown in the figure, push the door inwards.

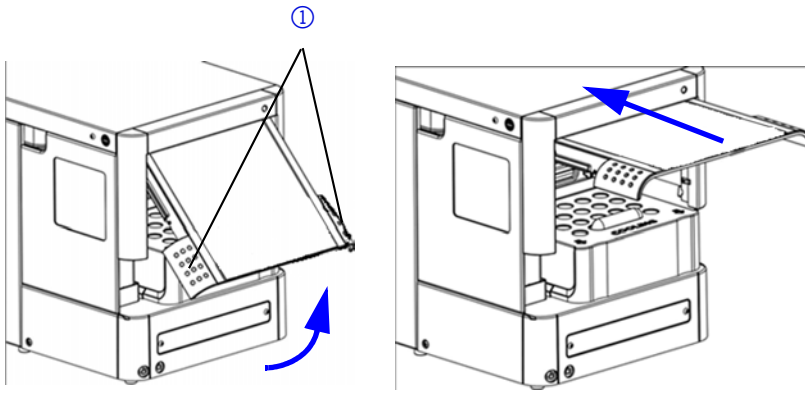



**Fig. 3 Push the door into the interior**

**Removing the front panel**

Press the buttons on the side ① at the same time and remove the front paneling.

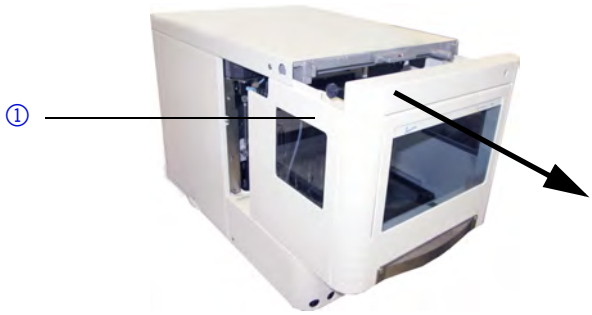



**Fig. 4 Removing the front panel**

**Removing the coolunit cover**

Pull the coolunit cover in the direction indicated by the arrow.

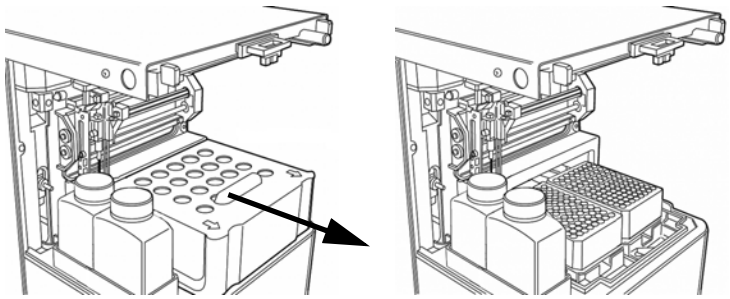


**Fig. 5 Removing the coolunit cover**

## Front View with Sample Compartment

**Legend**

① Valve

② Syringe

③ Needle guide

④ Collecting container

⑤ Flushing solution bottle

⑥ Removing the cooler cover

⑦ Tube connector for waste liquid

⑧ Tube connector for condensed water

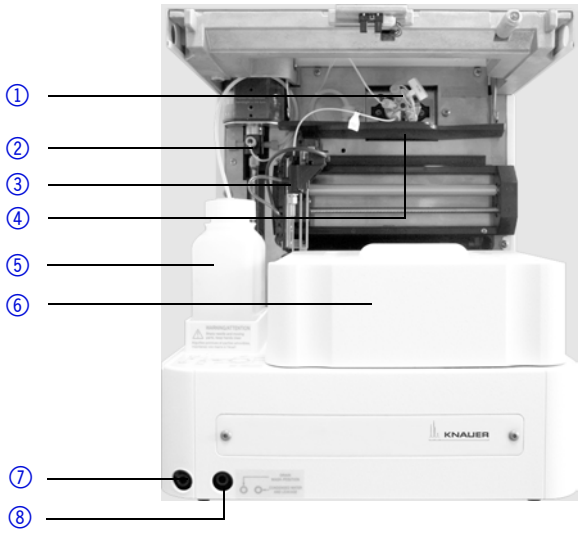



**Fig. 6 Front view with sample compartment**

# Rear View of the Device

The following components, connections and warnings can be found at the rear panel of the device:

**Legend**

① LAN port

② I/O connection (9-pin)

③ Warning 1

④ Power switch

⑤ Warning 2

⑥ CE mark

⑦ Fuse box

⑧ Mains power connection

⑨ Cooling fan

⑩ Serial number and year of manufacture of device

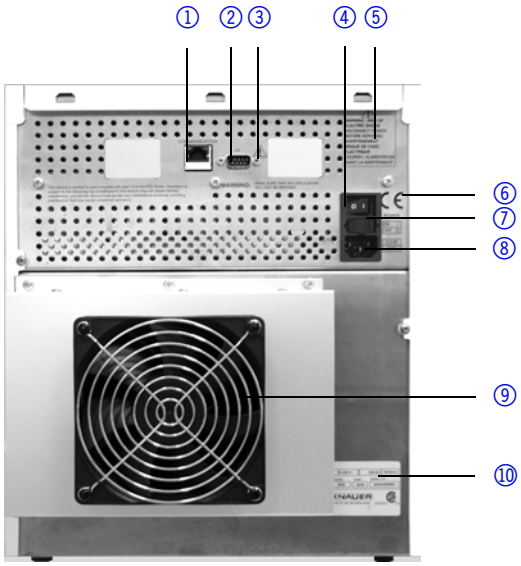



**Fig. 7 Rear panel of the device**

# Local Area Network and Automatic Configuration

The autosampler is operated exclusively using the chromatography software.

**Remote control** Normally, the pump is controlled by means of the chromatography software via a local network (LAN).

**Automatic configuration** The autosampler connected to the local area network (LAN) is automatically detected by the chromatography software.

**Device status** When used in a local area network (LAN), the system status of the autosampler can be checked using the chromatography software.

# Connecting the Device in a Local Area Network (LAN) to a Computer

This chapter describes how to set up a local area network (LAN) and how a network administrator can integrate this LAN into your company network. The description applies to the operating system Windows® and all conventional routers.

**Note** To set up a LAN, we recommend to use a router. The following steps are necessary:

**Process**

1. On the computer, go to the control panel and check the LAN properties.
2. Hook up the router to the devices and the computer.
3. On the computer, configure the router to set up the network.
4. Install the chromatography software from the data storage device.
5. Switch on the device and run the chromatography software.

## Configuring the LAN Settings

The LAN uses only one server (which is normally the router) from that the devices automatically receive their IP address.

**Prerequisite**

- In Windows®, power saving, hibernation, standby, and screen saver must be deactived.
- In case you use an USB-to-COM box, the option "Allow the computer to turn off ths device to save power" in the device-manager must be deactivated for all USB hosts.
- Only for Windows 7: For the network adapter, the option "Allow the computer to turn off ths device to save power" in the devicemanager must be deactivated.

**Procedure**

1. In Windows 7 choose *Start* ⇒ *Control Panel* ⇒ *Network and Sharing Center*.
2. Double-click on *LAN Connection*.
3. Click on the button *Properties*.
4. Select *Internet Protocol version 4 (TCP/IPv4)*.
5. Click on the button *Properties*.

6. Check the settings in the tab *General*. The correct settings for the DHCP client are:
   a) *Obtain an IP address automatically*
   b) *Obtain DNS server address automatically*
7. Click on the button *OK*.

## Connecting the Cables

A router has several LAN ports and one WAN port that can be used to integrate the LAN into a wide area network (WAN), e. g. a company network or the Internet. On the other hand, the LAN ports serve to set up a network from devices and a computer. To avoid interference, we recommend to operate the HPLC system separate from the company network.

**Note** You will find patch cables for each device and the router in the accessories kit. To connect the router to a WAN, an additional patch cable is required, which is not supplied within the scope of delivery.

**Legend**

① Modules
② Router
③ LAN port
④ WAN port
⑤ Workstation

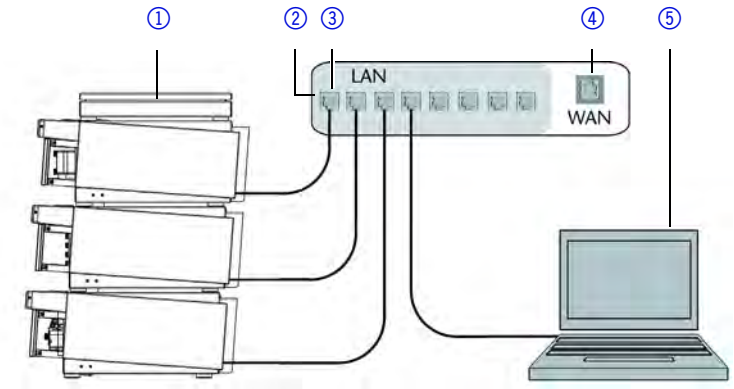



**Fig. 8 Cabling system LAN**

**Prerequisite**

- Computer is on.
- There is a patch cable for each device and the computer.

**Procedure**

1. Use the patch cable to connect the router and the computer. Repeat this step to connect all devices.
2. Use the power supply to connect the router to the mains power system.

## Configuring the Router

The router is preset at the factory. You can find a label at the bottom side of the router, on which IP address, user name, and passwort are printed. These information help to open the router configuration.

**Procedure**

1. To open the router configuration, start your Internet browser and enter the IP address (not for all routers).
2. Enter user name and passwort.
3. Configure the router as DHCP server.
4. In the router configuration, check the IP-address range and make changes if necessary.

**Result** Once the router has assigned IP addresses to all devices, the chromatography software can be used to remotely control the system.

### Integrating the LAN into a Company Network

A network administrator can integrate the LAN into your company network. In this case you use the WAN port of the router.

**Prerequisite** There is a patch cable for the connection.

**Procedure**

1. Check that the IP-address range of the router and of the company network do not overlap.
2. In case of an overlapping, change the IP-address range of the router.
3. Use the patch cable to connect the router WAN port to the company network.
4. Restart all device, including the computer.

### Controlling Several Systems Separately in a LAN

Devices connected to a LAN communicate through ports, which are part of the IP address. If more than one HPLC system is connected to the same LAN and you plan on controlling them separately, you can use different ports to avoid interference. Therefore, the port number for each device must be changed and this same number must be entered into the device configuration of the chromatography software. We recommend to use the same port number for all devices in the same system.

**Note** The port is set to 10001 at the factory. You must use the same numbers in the device configuration of the chromatography software as in the device, otherwise the connection fails.

**Procedure**

1. Change the port number of the device.
2. Enter the port number in the chromatography software.

**Result** The connection is established.

### Troubleshooting for Connection Problems

In case no connection between the computer and the devices can be established, go through the following points. Check after each point, if the problem is solved. If you did not manage to locate the problem, call the Technical Support.

| | |
|---|---|
| 1. Check the status of the LAN connection in the Windows taskbar:<br>- Connected<br>- Connection not established<br>If no connection was established, test the following:<br>▪ Is the router on?<br>▪ Is the patch cable connected correctly to the router and the computer? | ☐ |

| | |
|---|---|
| 2. Check the router settings:<br>▪ Is the router set to DCHP server?<br>▪ Is the IP-address range sufficient for all the connected devices? | ☐ |
| 3. Check all connections.<br>▪ Are the patch cable connected to the LAN ports and not the WAN port?<br>▪ Are all cable connections between devices and router correct?<br>▪ Are the cables plugged in tightly? | ☐ |
| 4. If the router is integrated into a company network, pull out the patch cable from the WAN port.<br>▪ Can the devices communicate with the computer, even though the router is disconnected from the company network? | ☐ |
| 5. In case you own a Control Unit, check the settings in the menu *Setup* > *Network*.<br>▪ Is *LAN-DHCP* set for controlling?<br>▪ Did the device receive an IP address? | ☐ |
| 6. Turn off all devices, router, and computer. Firstly turn on the router, secondly the devices and the computer.<br>▪ Has this been successful? | ☐ |
| 7. Replace the patch cable to the device with that no connection could be established.<br>▪ Has this been successful? | ☐ |

# PASA™ Loop Injection Principle

The autosampler uses loop injection with pressure assistance (Pressure Assisted Sample Aspiration PASA™) and has the following features:

- Samples do not have to be degassed
- No air bubbles in sample loop
- No clogging or contamination of sample needle
- Precise control of syringe movement

**Legend**

① Sample loop

② Syringe

③ Sample vial

④ Pump

⑤ Column

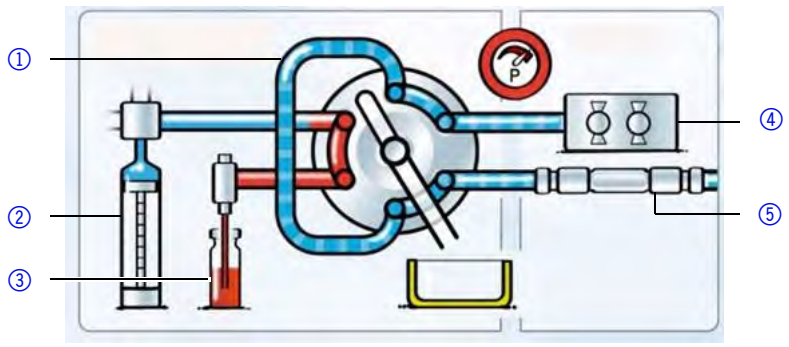



**Fig. 9 PASA™ loop injection principle**

## Injection Methods

Three different injection methods are available:

- Full loop injection
- Partial loop injection
- Microliter pick-up injection

**Full loop injection**

In full loop injection mode, the sample loop is completely filled with the sample. The maximum reproducibility but not the maximum precision is achieved because the size of the sample loop may have a deviation of ±10 %. The maximum injection volume equals the loop volume. The sample loop is filled with a multiple of the loop volume:

3 x loop volume for loops up to 100 µl

The sample loss per injection is the sum of the overfilling of the sample injection times x and the flush volume set for the needle used.

**Partial loop injection**

In partial loop fill mode, the sample loop is filled with both sample and eluent. This ensures the highest precision of the sample volume with minimal loss of sample. The maximum injection volume equals 50 % of the loop volume. The sample loss per injection equals the flush volume set for the needle used.

**Microliter pick-up injection**

In microliter pick-up injection mode, the sample loop is filled with a very small amount of sample and eluent (mobile phase). This ensures very high precision with no loss of sample. The

maximum sample volume is at 50 % of the loop volume minus 1.5 times the needle volume.

**Air needle and sample needle**

The autosampler uses a system of two telescopic needles, one that pierces through the cap of the sample vial – the air needle – and one that extracts the sample – the sample needle.

Using a syringe, the sample is aspirated through the two needles out of the sample vial while under pressure and into the sample loop. To prevent the syringe from becoming contaminated, a buffer tube is situated between the syringe and the valve. Using washing solution, sample residue is removed from the sample needle and buffer tube.

# Full Loop Injection

**Note** A flushing process takes places after every injection.

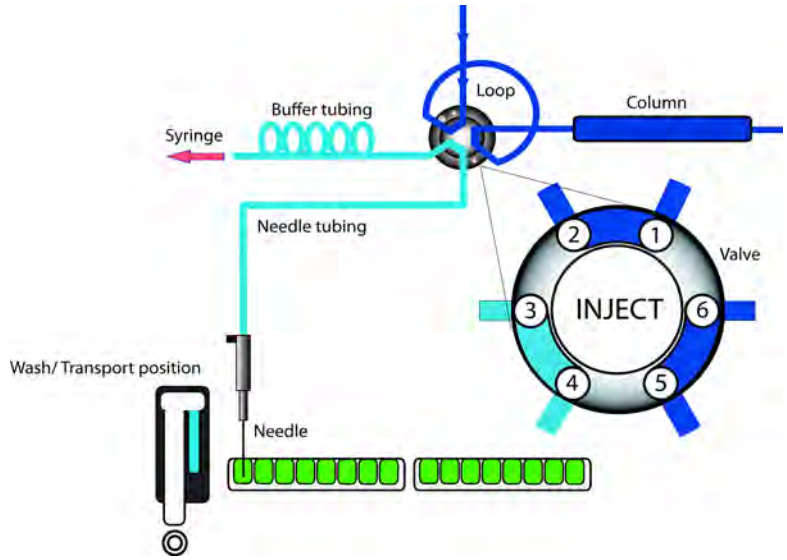
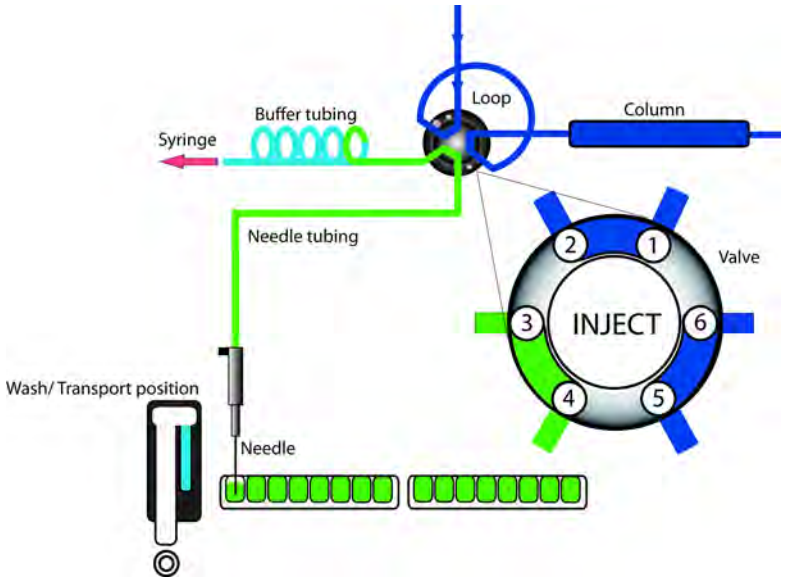
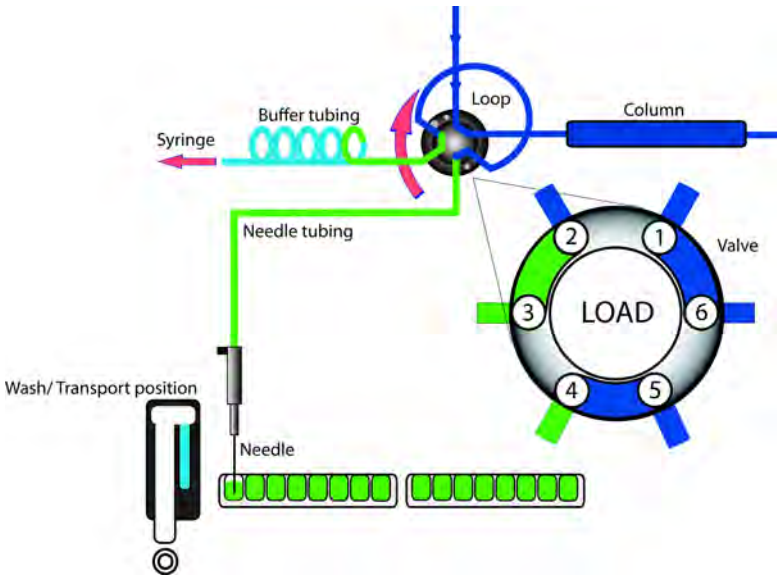
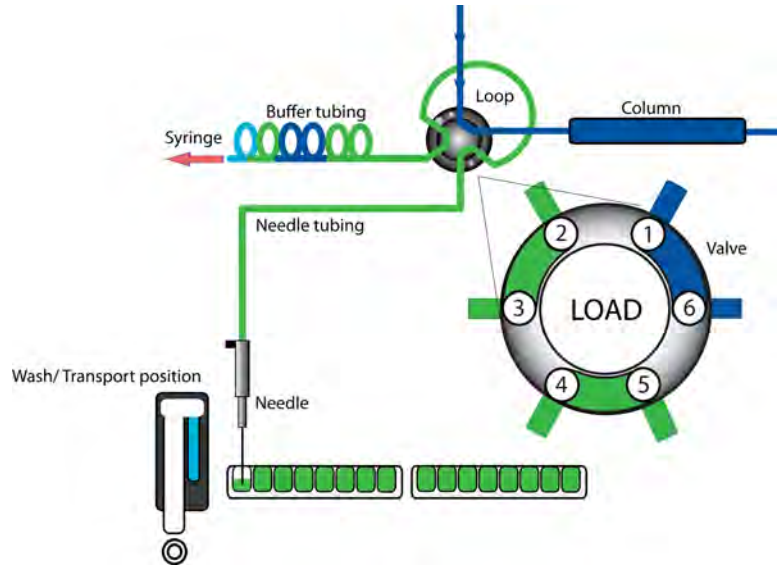
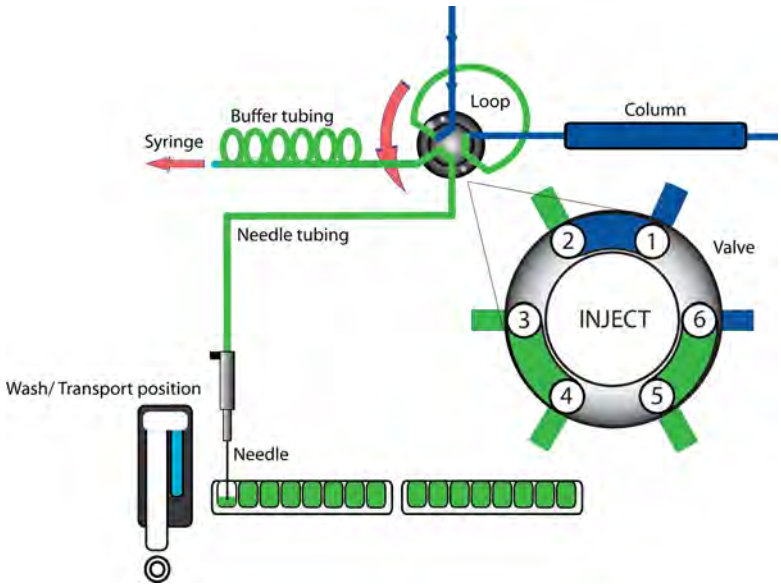
| Functional schematic | Explanations |
|---|---|
|  | 1. Initial conditions: The valve switches to the INJECT position. Sample needle and air needle are inserted into the vial. The air needle creates pressure, which prevents air and steam bubbles from developing. |

**Fig. 10 Full loop injection: Initial conditions**

| Functional schematic | Explanations |
|---|---|
|  **Fig. 11 Full loop injection: The needle and sample lines are flushed** | 2. The syringe aspirates the flush volume from the sample vial into the sample line and removes any washing solution. |
|  **Fig. 12 Full loop injection: Valve switches to *LOAD* position** | 3. The valve switches to LOAD position in order to transport the sample mateiral to the inlet of the sample loop. |

| Functional schematic | Explanations |
|---|---|
|  **Fig. 13 Full loop injection: The sample loop is fully filled.** | 4. The sample loop is filled by transporting a certain amount of the loop volume (depending on the volume of the loop) through the loop.<br>3 x loop volume for loops up to 100 µl |
|  **Fig. 14 Full loop injection: Valve switches to *INJECT* position** | 5. The valve switches to the INJECT position. From that moment, the sample loop is part of the HPLC flow of the mobile phase. The sample is transported to the column. The analysis starts. |

**Next steps** Flush the needle after every injection.

## Air Segment for Full Loop Injection

You can use an air segment of 5 µl to reduce the flush volume. The air segment precedes the flush segment and is not injected.

The flush volume must be at 30 µl in case of a standard needle and an injection with air segment; in case of an injection without air segment, the flush volume has to be 35 µl. You may

need to insert a higher flush volume for extremely viscous samples to reduce the syringe speed and improve the performance.

**Fig. 15 Air Segment for full loop injection**

# Partial Loop Fill

**Flush volume**

An air segment of 5 µl preceding the flushing volume, which is not injected, can reduce the amount of sample dilution caused by the dispersion during aspiration.

- Choose the following flush volumes for a standard needle:
  - At least 30 µl for injections with air segment
  - 35 µl for injections without air segment
- Increase the flush volume and reduce the syringe speed to achieve better results for highly viscous samples.

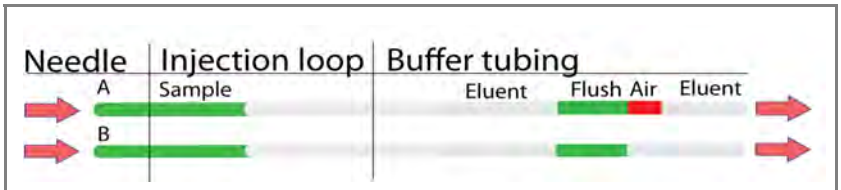



**Fig. 16 Air segment for partial loop full**

**Sample Volume** In the Autosampler 3950, the syringe moves the sample into the sample loop. In partial loop fill mode, the sample volume is maximum 50 % of the loop volume.

**Legend**

① Flushing solution

② Tube connector for waste

③ Syringe

④ Tube

⑤ Valve

⑥ Sample loop

⑦ Column

⑧ Needle

⑨ Microtiter plate with samples

⑩ Capillary

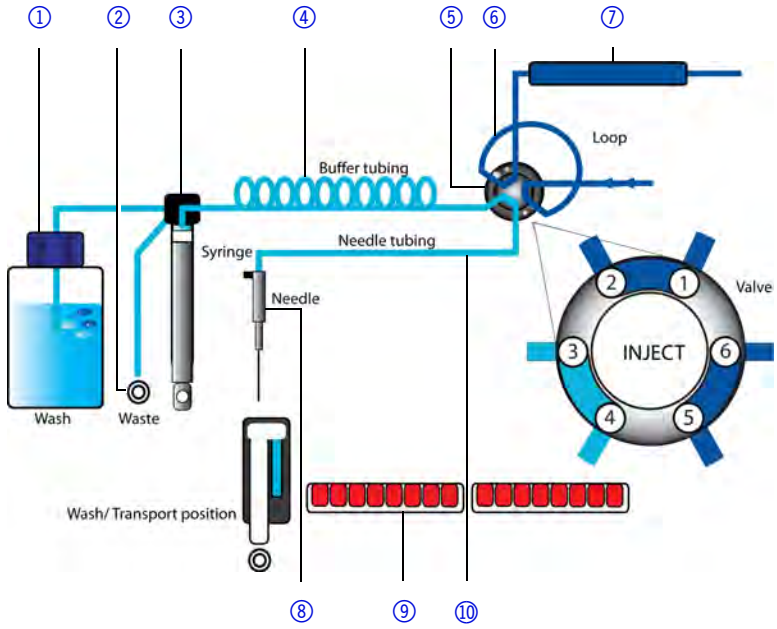
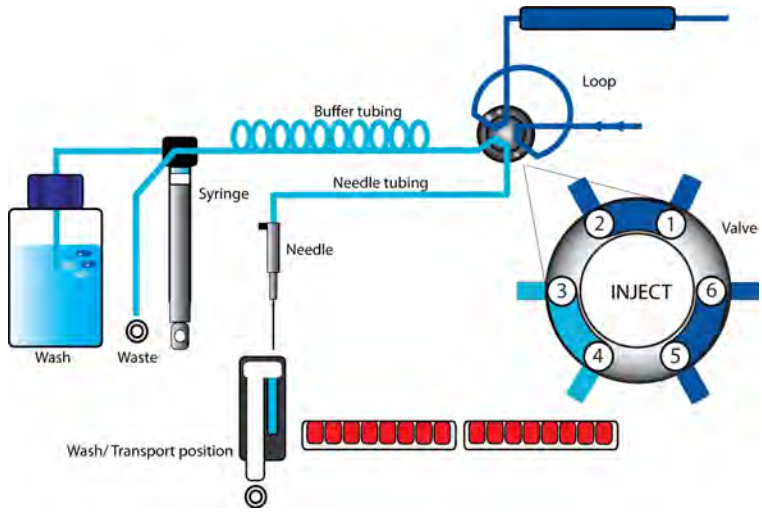
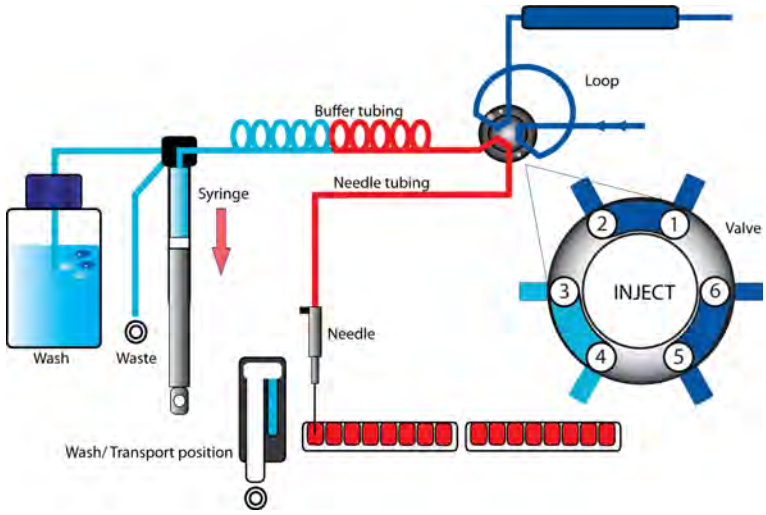
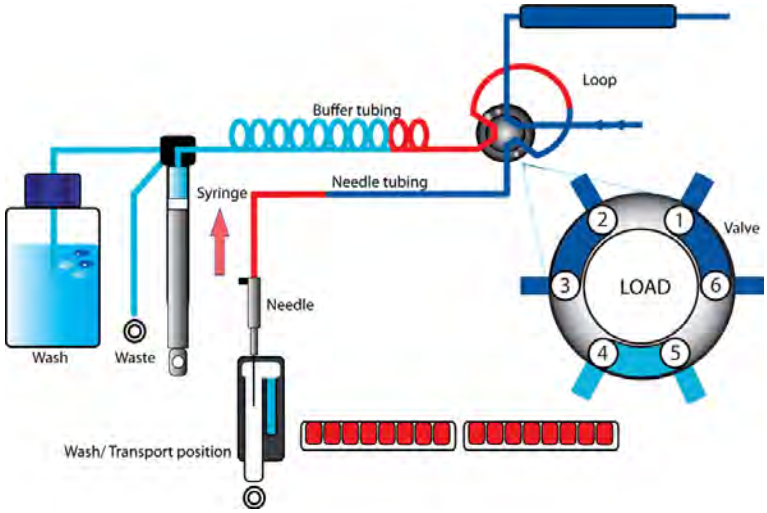
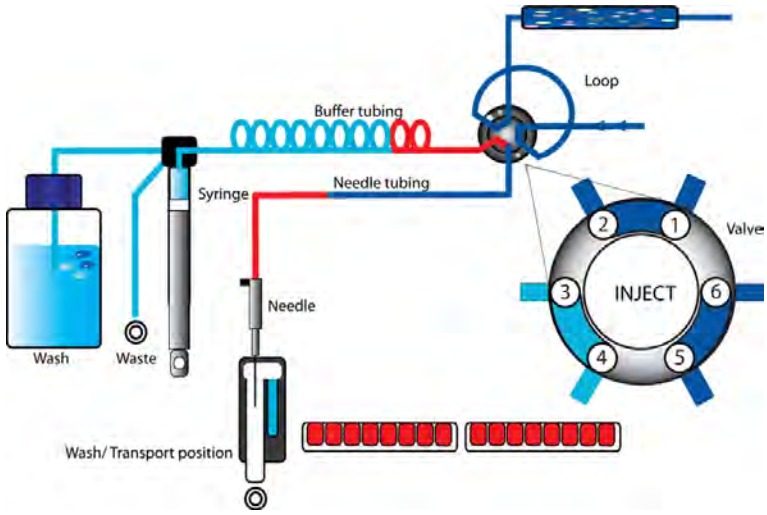
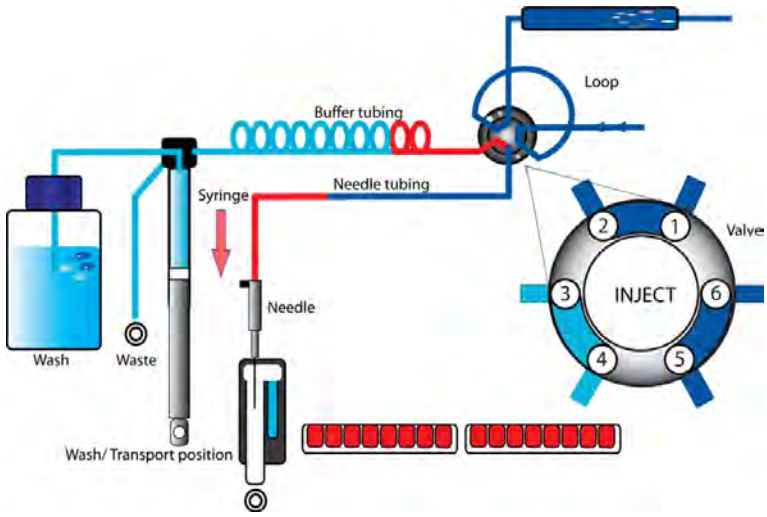
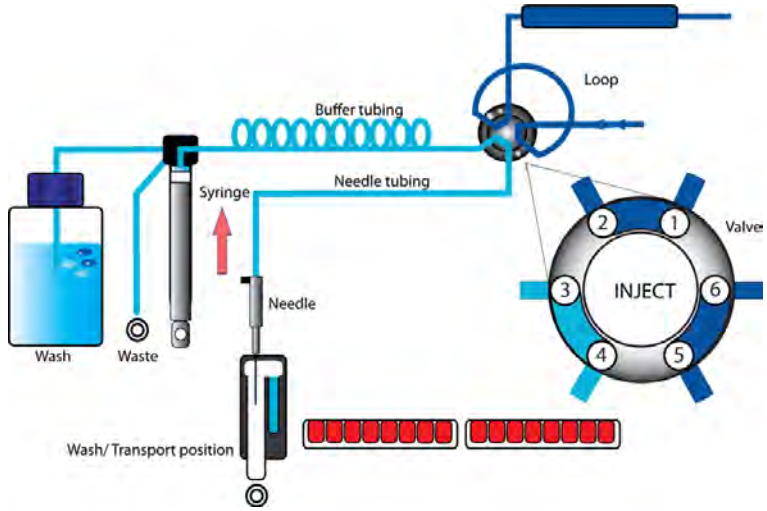



**Fig. 17 Scheme of initial position for partial loop fill**

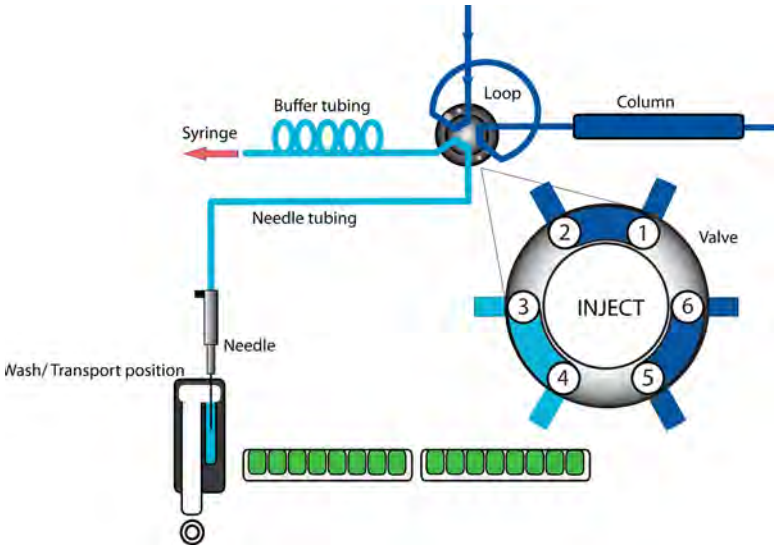
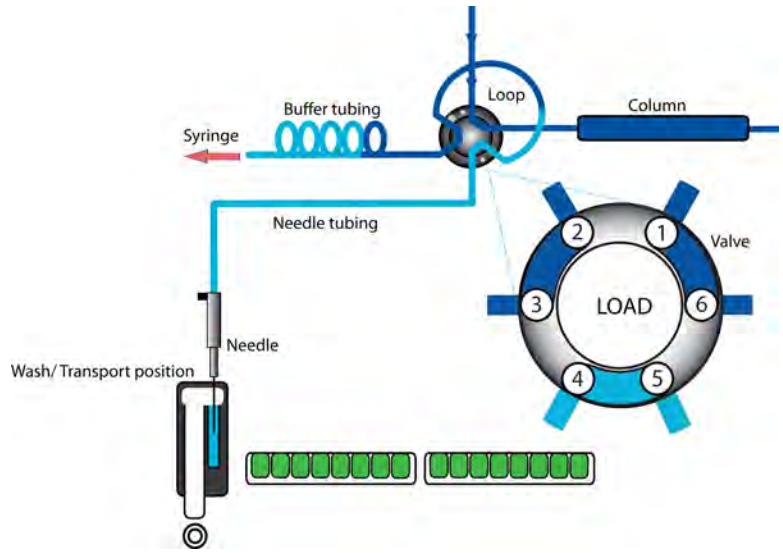
**Note** The partial loop fill is processed automatically.

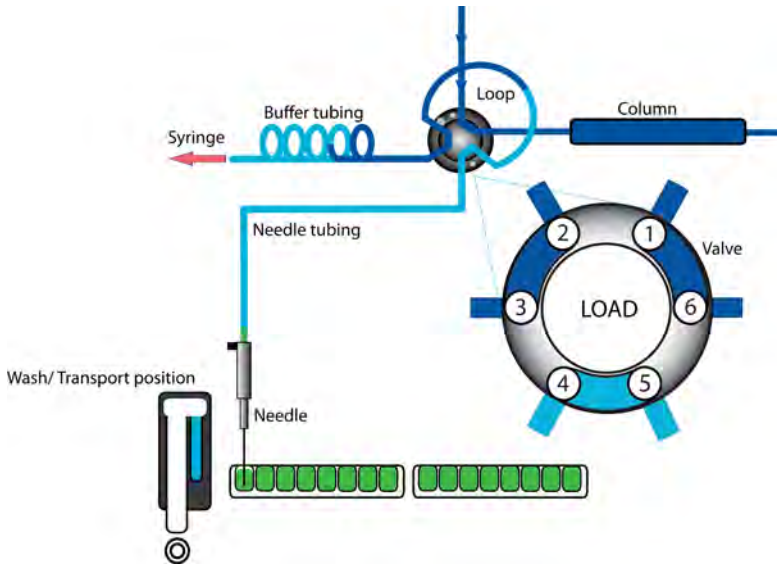
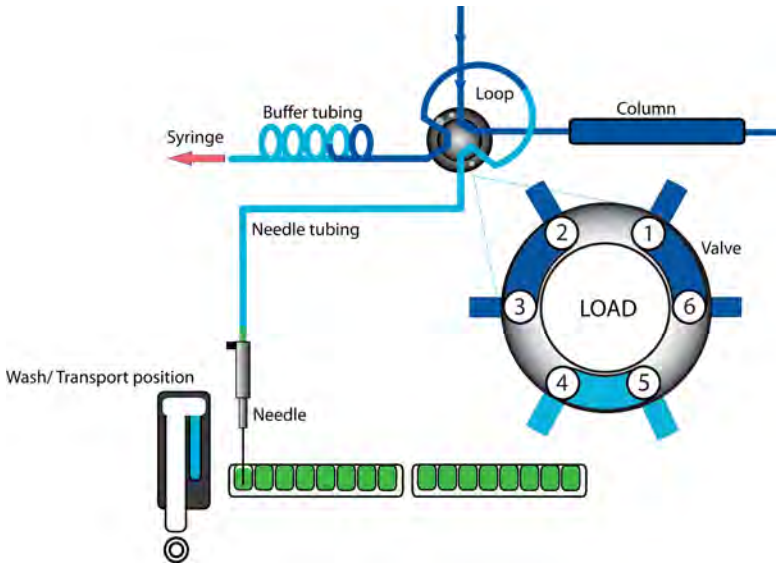
| Figure | Process |
|---|---|
|   **Fig. 18 Partial loop fill: Valve position "Inject"** | 1. The valve switches to the *INJECT* position. The tube and the capillary are filled with flushing solution. |

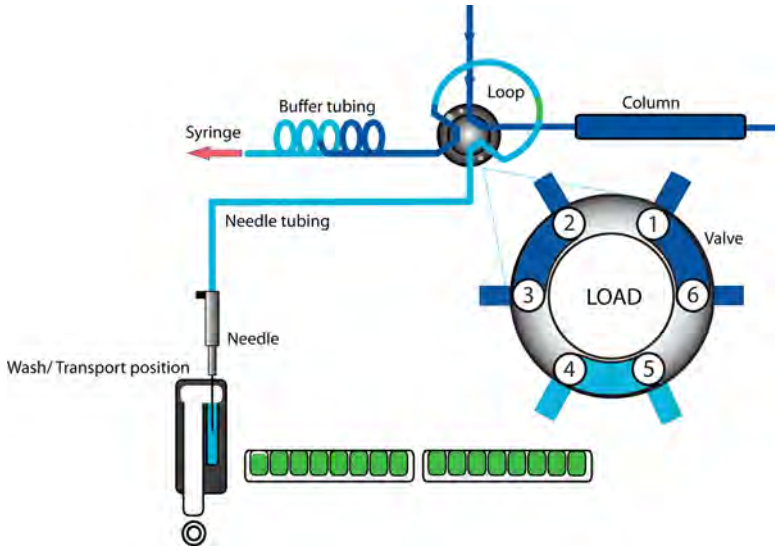
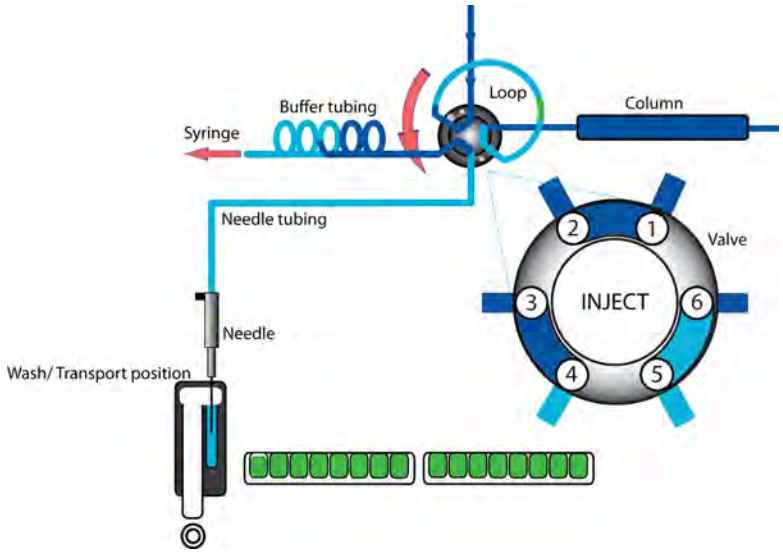
| Figure | Process |
|---|---|
|  **Fig. 19 Partial loop fill: Aspirating the sample** | 2. The needle moves into the sample and the syringe aspirates the sample. The tube is partially and the capillary fully filled with sample. |
|  **Fig. 20 Partial loop fill: Valve position "Load"** | 3. The valve switches into the *LOAD* position and the syringe aspirates the sample into the sample loop. The tube is full of flushing solution and sample, the capillary of sample and eluent. |
|  **Fig. 21 Partial loop fill: Injecting into the column** | 4. The valve switches to the *INJECT* position and the pump transports the sample and the eluent to the column. |

| Figure | Process |
|---|---|
|  **Fig. 22 Partial loop fill: Aspirating flushing solution** | 5. The syringe aspirates the flushing solution from the bottle. |
|  **Fig. 23 Partial loop fill: Flushing sample and eluent** | 6. The syringe injects the flushing solution into tube and capillary. Both sample and eluent get flushed. |

# Microliter Pick-Up Injection

| Figure | Process |
|---|---|
|  **Fig. 24 Microliter pick-up injection: Initial conditions** | 1. Initial conditions: The valve switches to the INJECT position. The sample needle is in position wash/transport. The transport container is filled with washing liquid.<br>2. The sample line is filled with washing fluid until the fluid reaches the sample loop inlet. The valve remains in the INJECT position during filling/ transportation.<br>**Note:** Make sure that washing liquid and eluent are compatible. |
|  **Fig. 25 Microliter pick-up injection: Sample line is filled with eluent** | 3. The valve switches to LOAD. A washing fluid segment is aspirated into the sample loop. |

| Figure | Process |
|---|---|
|  **Fig. 26 Microliter pick-up: Sample is aspirated** | 4. The needle moves from the transport position to the sample vial. |
|  **Fig. 27 Microliter pick-up: The injection volume is aspirated.** | 5. The sample is aspirated from the sample vial according to the amount of programmed injection volume. |

| Figure | Process |
|---|---|
|  **Fig. 28 Microliter pick-up: The sample is transported through the sample loop.** | 6. The needle moves back into transport position. A second washing liquid sample is aspirated. The sample is transported through the sample loop. |
|  **Fig. 29 Microliter pick-up: The sample is transported to the column.** | 7. The valve switches to INJECT. At that moment, the sample loop is part of the HPLC flow of the mobile phase. The sample is transported to the column. The analysis starts. |

## Air Segment with µl-Pick-Up Injection

If an air segment has been programmed, it will be inserted in front of the eluent area and in front of each sample.

The following conditions apply:

- The air segment is injected into the HPLC system in front of the sample segment.

- In this mode, the needle does not create pressure because the sample volume could be distorted due to the air expansion during the movement from sample vial to wash position.

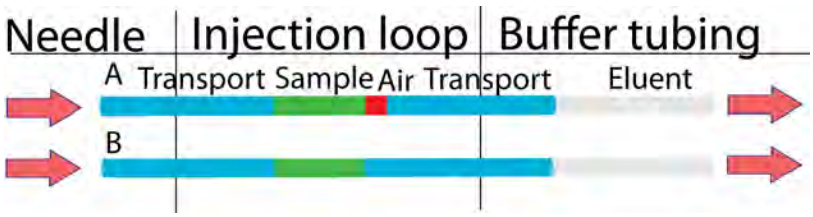
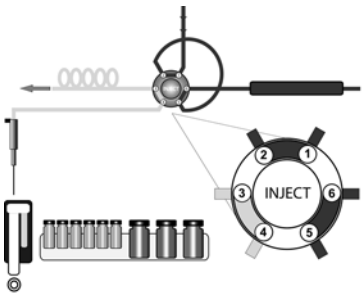
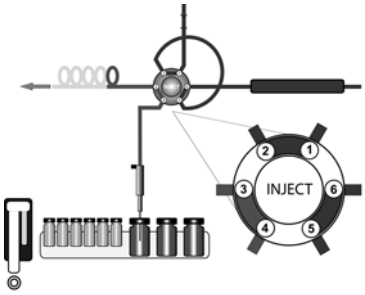
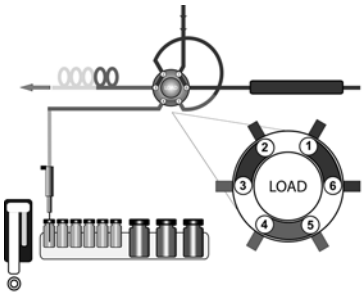
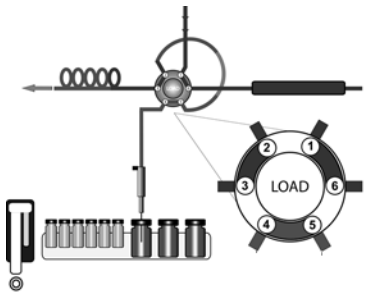
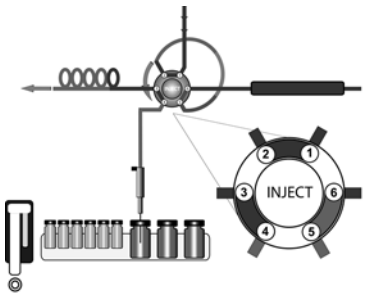



**Fig. 30 Microliter pick-up injection with air segment (A), without air segment (B)**

# Microliter Pick-Up Injection with 84+3

If you choose to operate the microliter pick-up with the 84+3 vial plate, three 10 ml vials are used automatically for transportation. In this case, the needle position wash/transport is only used to wash the needle. The following injection process is the result:

| Process | Figure |
|---|---|
| 1. The sample needle begins with the wash/ transport position. The valve starts with the INJECT position. |   **Fig. 31 Microliter pick-up 84+3: Initial conditions** |
| 2. The first injection starts in the transport position with the syringe aspirating transport liquid from the vial to fill the sample line with transport liquid and to remove the flushing solution. |   **Fig. 32 Microliter pick-up 84+3: Sample line is filled with flushing solution.** |

| Process | Figure |
|---|---|
| 3. The valve switches to LOAD. The programmed injection volume is aspirated out of the sample vial. |  **Fig. 33 Microliter pick-up 84+3: Injection valve switches to *LOAD* position** |
| 4. The needle moves back into the transport position. A second washing liquid segment is aspirated. The sample is transported through the sample loop. |  **Fig. 34 Microliter pick-up 84+3: The sample is transported through the sample loop.** |
| 5. The valve switches to INJECT. From that moment, the sample loop is part of the HPLC flow of the mobile phase. The sample is transported to the column. The analysis starts. |  **Fig. 35 Microliter pick-up 84+3: Injection valve switches to position *INJECT*.** |

## Air Segment with µl-Pick-Up Injection 84+3

If an air segment has been programmed, it is inserted in front of the transportation liquid segment and in front of each sample.

Details on this injection mode:

- The air segment is injected into the HPLC system in front of the sample segment

- In this mode, the needle does not create pressure because the sample volume could be distorted due to the air expansion during the movement from sample vial to wash position

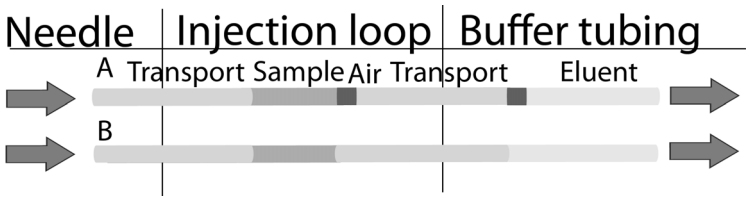



**Fig. 36 Microliter pick-up injection 84+3 with air segment (A), without air segment (B)**

# 84+3 Vial Plate

The 84+3 vial plate is to be ordered separately. Position the plate onto the plate holders inside the autosampler.

Hinweis: Position 87 of the vial plate must always be located at the rear right corner of the sample extraction chamber.

The vial plate offers space for maximum 84 vials with 1.5 ml and 3 vials with 10 ml. It was designed with the caps of all vials being level, independently from their unique heights, which means that one needle can be used for all vial sizes.

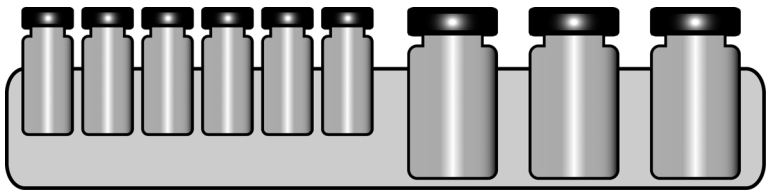


**Fig. 37 Height of 84+3 vial plate**

The length of the sample needle must be programmed at 1.5 ml. Relating to the liquid level in 10 ml vials, it is possible to program the needle at two different lengths.

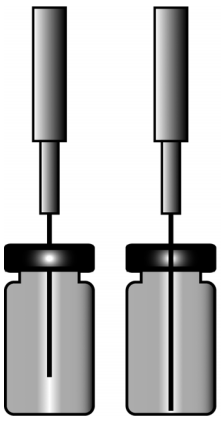


**Fig. 38 Needle length and liquid level**

Follow the numeric order if you are to program a sample sequence for a vial plate.

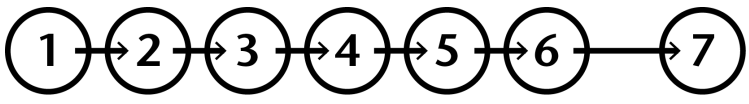



**Fig. 39 Sequence**

In case you are creating a sequence chart with only one sample per row (1 sample in each run), programming the sequence is free to you.

# µl-Pick-Up Parameters for the 84+3 Vial Plate

Before using the 84+3 vial plate, it is necessary to change the software settings.

**Note** If you choose to operate the microliter pick-up with the 84+3 vial plate, three 10 ml vials are used automatically for transportation. In this case, the needle position wash/transport is only used for washing the needle.

The vial positions are as follows for the 84+3 vial plate:

| | |
|---|---|
| Position of the first sample: | Vial positions 1-84 |
| Position of the final sample: | Vial positions 1-84 |
| First destination position: | Vial positions 1-84 |
| Transport position: | Vial positions 85-87 |

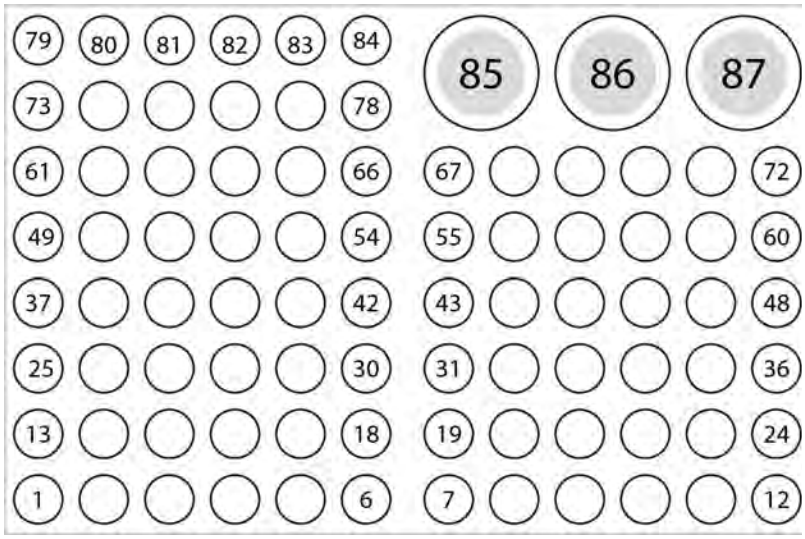



**Fig. 40 Reagent/Transport position on 84+3 vial plate**

You need to define the first and the final sample to enter a sample range. The transport positions are always positions 85, 86, 87.

## Details on Programming the 84+3 Injection Method

- The position of the transport vial can be programmed. Some possible positions are 85, 86, and 87. Position 85 is the standard position for transport vials. The system calculates the necessary transport volume. Make sure before system start that the vial is filled with at least 8000 µl of liquid.
- The liquid levels of the transportation liquids are not updated at system start. To avoid contamination of the air needle, the needle stops inside the transport vial in the up most position.

- If conducting a sample sequence or one sample per row, the autosampler takes the residual volume of the transport liquid into account. If the volume falls below 4000 µl, the needle moves deeper into the transport vial. If the amount of transport liquid reaches 0 µl, the autosampler sends out error message 369 (not enough transport liquid available).
- The liquid levels of the transportation liquid will reset after reprogramming the mode.
- The needle does not move automatically to the next transport vial. If transport liquid is to be taken from another position, you have to change the program settings.

# Air Needles

Six different lengths of air needles from 50 to 80 mm are available for the autosampler. The needle holder allows you to further adjust the needle height by 6 mm.

## Standard Air Needle

The standard air needle is 62 mm long and can be used for a wide range of deep and shallow vial plates.

When 10 ml sample vials are used, the needle deeply penetrates the sample vial. If this is not filled to more than 60%, the needle can be used in the typical manner. The same applies to deep microtiter plates.

For non-standard settings, use the corresponding needle types.

**Legend**

① 10 ml sample vial

② 2 ml sample vial

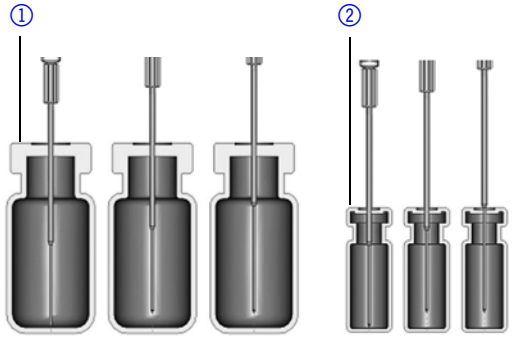



**Fig. 41 Standard air needle with 10 and 2 ml sample vial**

**Note** The PASA™ loop injection principle is not suitable for shallow microtiter plates. The function of the air needle is only ensured when it pierces the closure to a sufficient degree.

**Legend**

① Deep Greiner Bio-One® microtiter plate with closure.

② Shallow Greiner Bio-One® microtiter plate

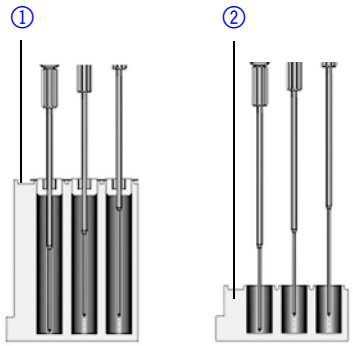



**Fig. 42 Standard air needle with Greiner Bio-One® microtiter plates**

# Choosing the Correct Air Needle

To choose the correct air needle, take the following dimensions into consideration:

**Legend**

$H_t$ = height of vial plate

$D_w$ = hole depth
$C_d$ = thickness of closure
$N_h$ = set needle height
$A_c$ = distance from air needle tip to closure (min. 2 mm)
**?** = excess length

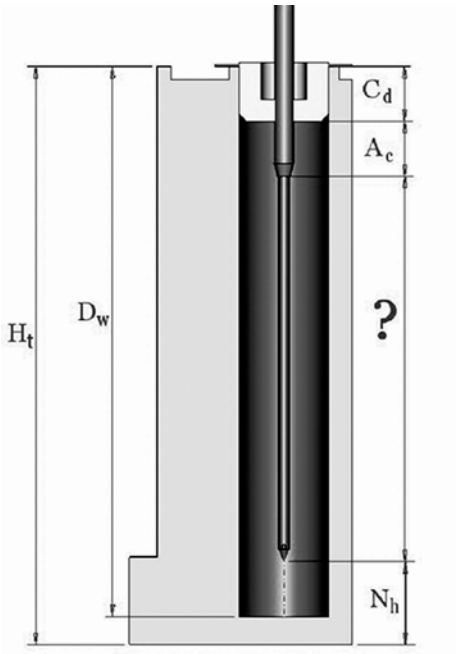



**Fig. 43 Calculating the correct air needle**

**Condition:** $H_t - D_w$ = 2 to 6 mm

Excess length of the sample needle:
$H_t - C_d - N_h - A_c = ?$

Choose the correct needle type on the basis of the excess length.

| Air needle type | Protrusion length |
|---|---|
| 50 mm, yellow | 34-40 mm |
| 56 mm, red | 28-34 mm |
| 62 mm, white (standard needle) | 22-28 mm |
| 68 mm, blue | 16-22 mm |
| 74 mm, green | 10-16 mm |
| 80 mm, black | 4-10 mm |

**Legend**

① 10 ml sample vial, 50 mm air needle

② 2 ml sample vial, 62 mm air needle

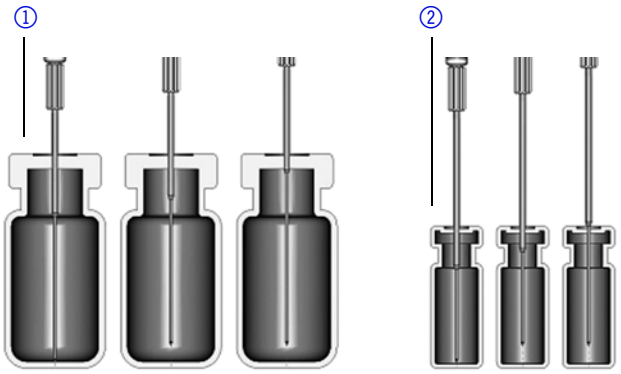



**Fig. 44 Selected air needles with sample vials**

**Legend**

① Deep Greiner Bio-One® microtiter plate with closure., 56 mm air needle

② Shallow Greiner Bio-One® microtiter plate, 80 mm air needle

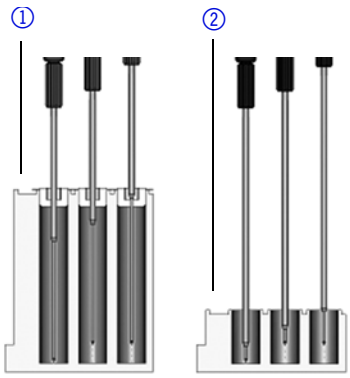



**Fig. 45 Selected air needles with Greiner Bio-One® microtiter plates**

### Calculation Example for Air Needle

Starting point:

- Autosampler with standard setting for needle height.
- Deep Greiner Bio-One® microtiter plate with closure.

Dimensions:

$\mathbf{H_t}$ = 41.4 mm

$\mathbf{D_w}$ = 37.8 mm

$\mathbf{C_d}$ = 3.8 mm

$\mathbf{N_h}$ = 6.0 mm (standard)

$\mathbf{A_c}$ = 2.0 mm (minimum)

Condition:

$\mathbf{H_t - D_w}$ = between 2 and 6 mm

$\mathbf{H_t - D_w}$ = 41.4 mm - 37.8 mm = 3.6 mm. Condition has been met.

$H_t - C_d - N_h - A_c$ = excess length

41.4 mm - 3.8 mm - 6.0 mm - 2.0 mm = 29.6 mm

| Air needle type | Protrusion length |
|---|---|
| 56 mm, red | 28-34 mm |

An air needle length of 56 mm is required.

## Handling the Sample Vials

When handling the sample vials, observe the following:

- Fill the sample vials using a pipette to allow air to escape.
- To prevent the sample from contaminating the air needle, do not fill the sample vials to the very top.
- Do not use sample vials that are unclosed.
- Only use air-tight closure seals to prevent air bubbles from forming and volatile components from evaporating.
- Do not use sample vials with hard closures that the sample needle cannot pierce.

# Mixing and Diluting

A mix method can be programmed for the autosampler to mix or dilute the sample fluid.

- Configure the mixing routine and syringe speeds using the chromatography software.
- A maximum of 15 steps can be programmed for a mix method.

Three types of actions are possible:

1. Add
2. Mix
3. Wait

**Add** When adding, the defined volume is aspirated from either the sample vial, the vial with *Reagent A* or *Reagent B* or flushing fluid and then dispensed into the destination vial.

**Note** To prevent carryover, the autosampler removes 125% of the given volume from the corresponding sample vial and uses the additional 25% to flush the tube and needle.

**Mix** When mixing, the contents of a specific sample vial is mixed by aspirating and dispensing the defined volume n times. If a destination vial has not been defined, mixing is performed in the current sample vial.

**Note** When entering the *Sample Vials*, the *Destination Vial* is used automatically.

**Wait** With the Wait command, the system waits until the programmed delay time has elapsed before executing the next line of the program.

## Example: Add

The command *ADD 100 µl from Reagent A to Destination* involves the following steps:

1. An air segment of 5 µl is aspirated to separate the flushing solution in the buffer tube from *Reagent A*.
2. 50 µl of *Reagent A* are aspirated to flush the tube and needle.
3. Syringe is emptied into the waste container through the drainage tube.
4. 100 µl of *Reagent A* are aspirated and then dispensed into the destination vial.
5. Tube and needle are flushed with flushing solution.

## Example: Mix

If this is preceded by an *ADD ... to Destination* command, mixing is performed in the destination vial. If this is preceded by an *ADD ... to Sample* command, mixing is performed in the sample vial.

The *MIX 3 times with 100 µl* command triggers the following steps:

1. An air segment of 50 µl is aspirated to separate the flushing solution in the buffer tube from the sample solution to be mixed.
2. Syringe is emptied into the waste container through the drainage tube.
3. 100 µl solution are aspirated and dispensed into the same sample vial.
4. Step 3 is repeated twice.
5. Tube and needle are flushed with flushing solution.

# Sample Positions in Mix Method

When configuring a mix method, the positions of the sample vials depend on whether the vial plates are to be processed in rows or columns.

## Processing in Columns

When column processing is used, the following positions are possible for the *Sample, Destination, Reagent A* and *Reagent B*:

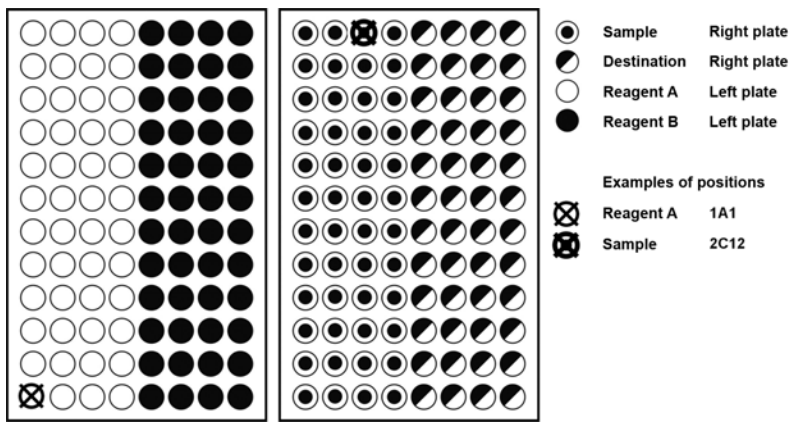



**Fig. 46 Sample positions in columns**

## Processing in Rows

When row processing is used, the following positions are possible for the *Sample, Destination, Reagent A* and *Reagent B*:

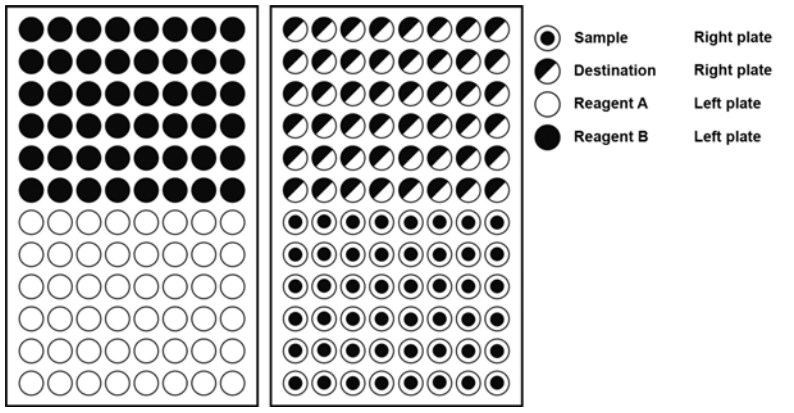



**Fig. 47 Sample positions in rows**

# Parameters for Mixing Method with 84+3 Vial Plate

Before using the 84+3 vial plate, it is necessary to change the software settings.

The vial positions for the 84+3 vial plate are as follows:

| | |
|---|---|
| Position of the first sample: | Vial positions 1-84 |
| Position of the final sample: | Vial positions 1-84 |
| First destination position: | Vial positions 1-84 |
| Reagent position: | Vial positions 85-87 |

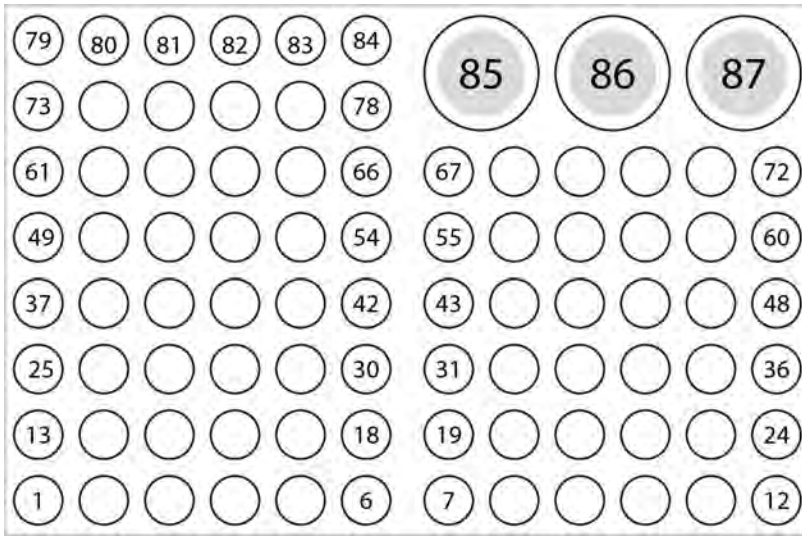



**Fig. 48 Reagent/Transport position on 84+3 vial plate**

You need to define the first and the final sample to enter a sample range. The same range can be used for the destination vials. The reagent positions are always positions 85, 86, 87.

## Details on Programming the 84+3 Mixing Method

- The position of the reagent vial can be programmed. Some possible positions are 85, 86, and 87. The standard transport positions are 86 for Reagent A and 87 for Reagent B. The system calculates the necessary reagent volume. Make sure before system start that the vials are filled with at least 8000 µl of liquid.
- The liquid levels of the reagent liquids are not updated at system start. To avoid contamination of the air needle, the needle stops inside the reagent vial in the up most position.
- If conducting a sample sequence or one sample per row, the autosampler takes the residual volume of the reagent liquid into account. If the volume falls below 4000 µl, the needle moves deeper into the reagent vial. If the amount of reagent liquid reaches 0 µl, the autosampler sends out error message 370 (not enough reagent liquid available).
- The liquid levels of the reagent liquid will reset after reprogramming the mode.
- Reagent will only be taken from preprogrammed positions. The needle does not move automatically to the next reagent vial. You have to change the settings when reagent is to be taken from one of the other vials.

# Capillary and Hose Connections

**Note** Flush the system before connecting the column.

The connection scheme inside the autosampler displays the correct connections:

**Legend**

① Waste hose

② Flushing fluid container

③ Buffer tube

④ Sample loop

⑤ Column

⑥ Pump

⑦ Injection needle

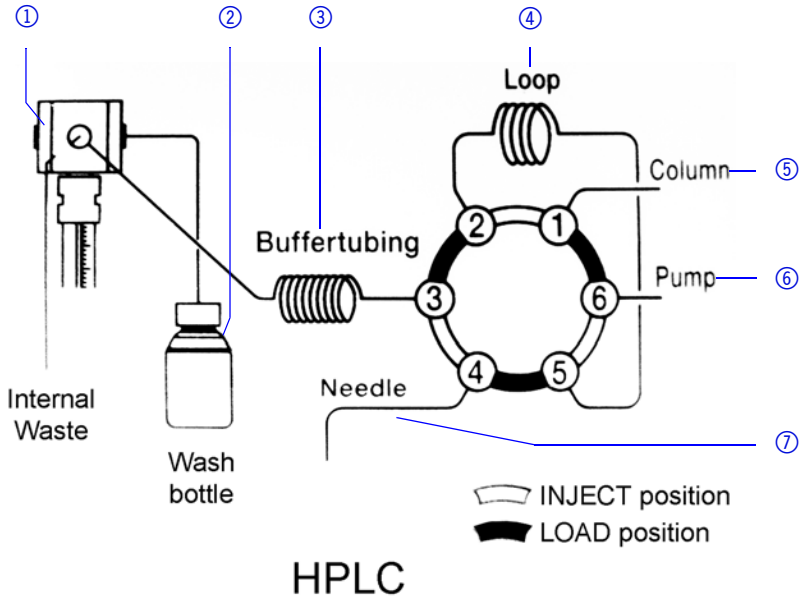



**Fig. 49 Connection scheme for capillaries and tubing**

## Connecting the Injection Valve

**Legend**

① Connection stainless steel capillary to column

② Connection sample loop

③ Connection tube buffer to syringe

④ Connection plastic capillary to injection needle

⑤ Connection sample loop

⑥ Connection stainless steel capillary to pump

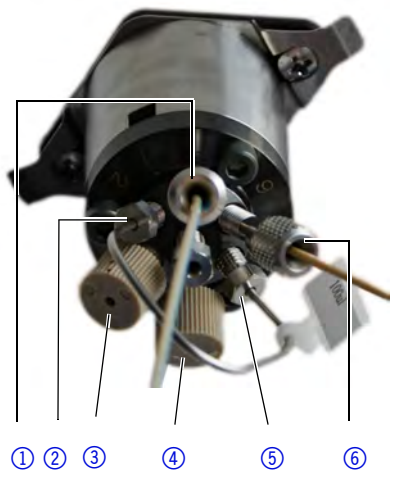



**Fig. 50 Connections on the injection valve**

# Connecting the Syringe

**Legend**

① Connection transport liquid

② Connection buffer tube

③ Connection flushing solution

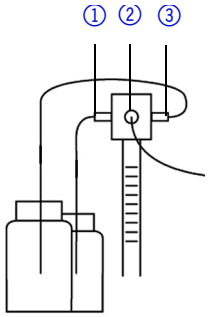



**Fig. 51 Syringe connections**

## Tube Guide for Flushing Solution

**Note** Use the tube guide in the collecting container for the flushing solution in order to not hinder horizontal movement of the needle.

**Legend**

① Tube Guide for Flushing Solution

② Collecting container with hole for tube guide (flushing fluid)

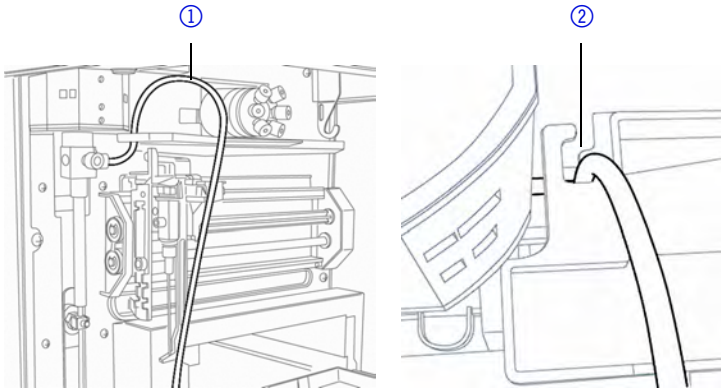



**Fig. 52 Tube guide for flushing solution tube**

# Connecting the Drainage Tubing

The waste drainage removes all flushing fluids and non-injected sample solutions.

- Connect the condensed water and drainage tubing to the front panel of the device.
- Connect the adapter and insert the end of the tube into a container on the floor.

**Note** Make sure that the drainage tubing is not pinched so that the liquid can drain.

**Legend**

① Tube for waste liquid

② Tube for condensed water

③ Adapter for drainage tube

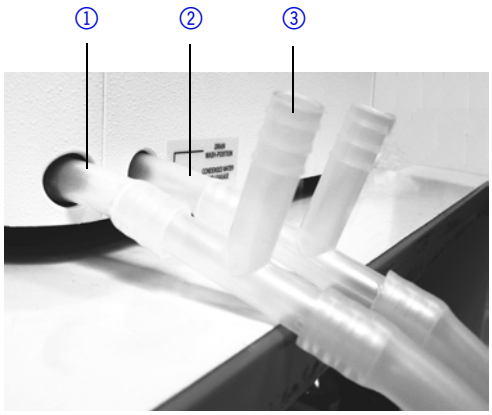



**Fig. 53 Connecting the drainage tubing**

# PEEK Connections

Disposable PEEK fittings

- One-piece disposable polyetheretherketone fittings (PEEK) for easier installation of flexible, thin capillaries (outer diameter 0.5 mm)
- Tightening torque of the PEEK screw:
  Tightened by hand (about 0.5 Nm)

# Connecting the Autosampler to Other Devices

## Controlling the Autosampler with Chromatography Software

You control the autosampler directly with software, e. g. ChromGate® and ClarityChrom® from KNAUER.

Using the LAN connection at the rear side of the device, connect the autosampler to the network.

### Checking and Configuring the Parameters of the Autosampler

You can configure the autosampler parameters using the chromatography software, e.g. ClarityChrom®:

1. Select the autosampler in LAN.
2. Set the syringe volume to either 250 µl (default) or 500 µl.
3. Enter the serial number of the autosampler.
4. Choose the cooling option when your autosampler offers temperature control.

### Configuration Window of ClarityChrom®

**Legend**

① Serial number

② Device detection in local network

③ Manual search for device in network

④ Volume of sample loop

⑤ Volume of syringe

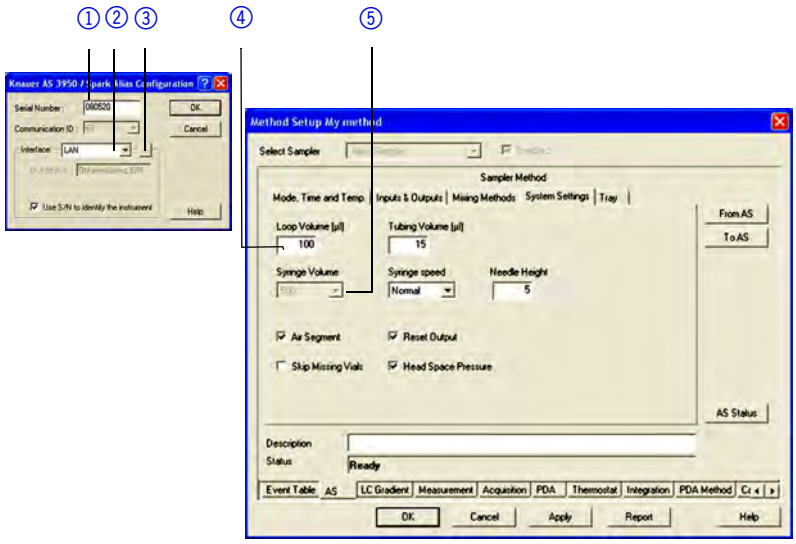



**Fig. 54 Autosampler configuration with ClarityChrom®**

# Autosampler Device Software

A software CD with *Autosampler 3950 Service Manager* is included with the autosampler. Using this software, you can check and control the device. These settings can also be made with the chromatography software, so that it is not absolutely necessary to install the *Service Manager*.

# Flushing the System

The system should be flushed before the column is connected. The flushing of the system can be controlled using the chromatography software or *Autosampler 3950 Service Manager*.

**Note** KNAUER recommends using a mixture of water and isopropanol (80 %/20 %) or the mobile phase as the flushing solution.

The following steps are explained for when the *Service Manager* is used.

## System Flushing with *Autosampler 3950 Service Manager*

1. Install *Autosampler 3950 Service Manager*.
2. Fill the flushing solution into a solvent bottle and degas it using helium or an ultrasonic bath.
3. Slide the tube for the coolant into the flushing solution bottle.
4. Select the *Alias* Þ ⇒*Direct Control* menu.
5. In the *Syringe* field, click *End*. One syringe volume is aspirated into the syringe through the flushing solution tube.
6. In the *Syringe* field, click *Home*. The syringe content is emptied into the drainage tube.
7. Repeat step 5 and 6 until the syringe and the flushing solution tube have been filled completely.
8. In the *Initial wash* field, click *Start*. All tubes that are connected to the syringe are flushed.
9. In the *Initial wash* field, click *Stop*.
10. Click *Close* to exit the *Direct Control* window.

**Practical tip!** Perform additional flushing to remove all of the air from the syringe.

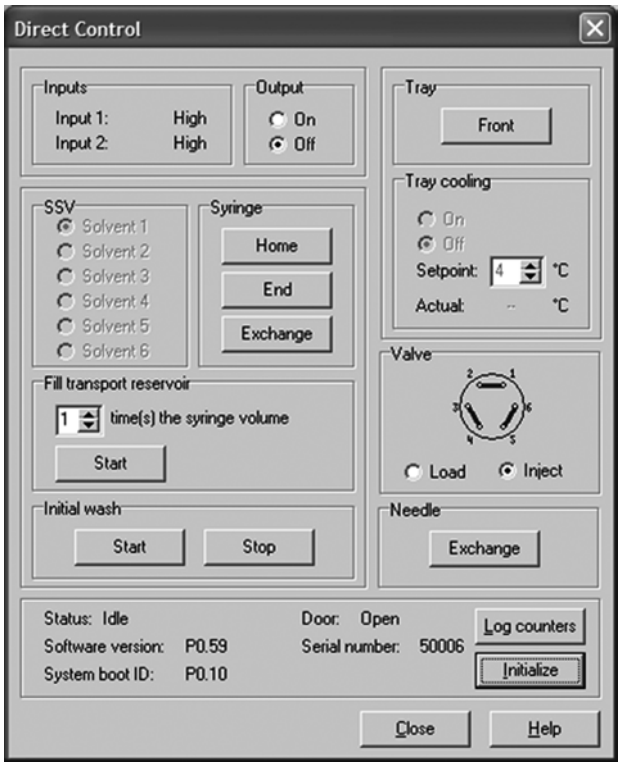



**Fig. 55 System flushing with the *Service Manager* software**

# I/O Connection

By default, the autosampler has an I/O connection that supports TTL inputs (low-active) and a closed-contact output. Devices without LAN connection that require a trigger signal for the injection can be connected by means of the I/O connection.

The TTL inputs allow you to control the autosampler using other devices and are defined using the instrument methods of the chromatography software.

**Note** Only connect the autosampler to devices that fulfill the required safety standards!

## Defining the TTL Inputs

- *Next Injection Input*: The injection sequence is started. After ending the injection sequence, the autosampler waits for the next start signal.
- *Freeze Input*: The analysis time is paused. The autosampler runs the configured program up to the filling of the sample loop. The injection is not performed until the input is deactivated.
- *Stop Input*: Immediately halts the analysis.

## Defining the Closed-Contact Output

- *Inject Marker*: The closed-contact output is activated when the injection valve switches from *LOAD* to *INJECT*.
- *Alarm*: The closed-contact output is activated when there is an autosampler fault.
- Auxiliary: -

## Configuration of I/O Connection (9 pins)

| Explanation | Cable color |
|---|---|
| 1. Output, start injection | Red in three-conductor cable |
| 2. Output, start injection | Black in three-conductor cable |
| 3. Input 1, programmable input for stopping injections (low-active) | Red in four-conductor cable |
| 4. Input 2, programmable input for stopping injections (low-active) | Black in four-conductor cable |
| 5. Not occupied. | - |
| 6. Output | Brown in three-conductor cable. |
| 7. Not occupied. | - |
| 8. Ground, for inputs 1 and 2 | Orange in four-conductor cable |
| 9. Ground, for inputs 1 and 2 | Brown in four-conductor cable |

# Device Test

The reproducibility of the sample volume is a critical factor for maintaining high-quality analysis results.

- Test the autosampler features with the chromatography software on a regular basis.
- If the device test determines that the autosampler does not fulfill the requirements, mark the device as defective and do not continue using it.
- Do not re-use the autosampler until it has been repaired and/or serviced.

## Test Intervals

Run the device test at the following time intervals:

- Average use of 1 to 5 days/week: Device test every 6 months
- Average use of more than 5 days/week or 24 hours/day: Device test every 3 months
- Operation with buffer solutions or other salt solutions: Device test every 3 months

## Devices and Components for the Test

- Autosampler 3950, standard version with 100 µl sample loop and 500 µl syringe
- HPLC pump, 1 ml/min flow rate
- UV detector (with a data rate of 50 Hz if possible, otherwise 10 Hz, flow cell: 10 mm path length)
- Chromatography software
- Eluent: 90 % water, 10 % methanol (HPLC quality)
- Test solution (sample):
  - *a:* 50 ppm uracil dissolved in water (HPLC quality)
  - *b:* 250 ppm uracil dissolved in water (HPLC quality)
- Flushing solution:
  - 80 % water, 20 % isopropanol (HPLC quality)
  - Alternative: 80 % water, 20 % methanol (HPLC quality)
- Restriction capillary:
  - Inner diameter 0.25 mm
  - Length 200 cm

**Note** Degas the eluent to prevent malfunctions caused by the presence of air bubbles.

# 1. Reproducibility of Sample Volume

The variation coefficient must not exceed 0.5 %.

- Configure the HPLC pump, UV detector and autosampler using the chromatography software
- Inject 10 µl of test solution *a* (50 ppm uracil, dissolved in water)

## Standard Setting of Autosampler

- Loop volume: 100 µl
- Tubing volume: 15 µl
- Syringe volume: 500 µl

## Method Parameters of Pump

- Flow: 1 ml/min
- Time: 1 min

## Method Parameters of Autosampler

- Injection method: Partial loopfill
- Syringe speed: normal
- Flush volume: 30 µl, (40 µl for microtiter plates)
- Needle wash: active 2 times
- Air segment: yes
- Headspace pressure: yes
- Injections/vial: 9 (7 for microtiter plates)
- Inj. volume: 2 µl
- Vial position: 1A1. Start the single run with

## Method Parameters of UV Detector

- Wavelength: 254 nm
- Sampling rate: 50 Hz if possible, else 10 Hz
- Time: 0.5 min

## Configuring Repeat Runs of Autosampler

- Injections/vial: 9x (7x for microtiter plates)
- Inj. volume: 10 µl

## Starting Repeat Runs

1. Put a vial with at least 500 µl test solution on position *1A1* of the sample plate.
2. Start the repeat runs.

### Analyzing the Individual Chromatograms

1. Calculate the average of the measuring values of the *Peak* areas.
2. Calculate the variation coefficient *VK1* .
3. Enter the results into the *Test Report* form.

### Formula for Determining the Arithmetic Mean

$$\overline{Peakareas} = \frac{\sum_{i=1}^{n} Peakareas_i}{n}$$

- Formula for determining the standard deviation (*i* = 1-9):

$$\sigma_{n-1} = \sqrt{\frac{\sum (Peakarea_i - \overline{Peakareas})^2}{n-1}}$$

- Formula for determining the variation coefficient:

$$VK_1 = \frac{\sigma_{n-1}}{peakarea} \times 100\,\%$$

## 2. Sample Carryover

The percentage of sample carryover must not exceed 0.3 %.

- Alternately inject 10 µl of test solution *b* (250 ppm uracil, dissolved in water) followed by eluent.
- Position of sample vial: *1A1*
- Position of eluent: *1A2*
- Injection volume: 10 µl

### Creating a Sequence with 6 Lines

- Test solution: Position *1A1*
- Eluent: Position *1A2*
- Injection volume: 10 µl
- Repeats: 1

**Note** For microtiter plates, select 6 consecutive positions that are alternately to be filled with test solution and eluent.

### Analyzing the Individual Chromatograms

1. Calculate the average of the measuring values of the *Peak* areas.
2. Put the average of the eluent injection in relation to the average of the test solution injection.
3. Enter the results into the *Test Report* form.

### Formula for Calculating Sample Carryover

$$PV = \frac{\sum_i \frac{Peakarea_{i\ Fließmittel}}{3}}{\sum_i \frac{Peakarea_{i\ Testlösung}}{3}} \times 100\ \%$$

## 3. Linearity

To determine the linearity, the correlation coefficient of the regression lines is determined from the measured values for the *peak* areas and injection volume.

The correlation coefficient must not exceed 0.998 %.

- Inject 10, 20, 30, 40 and 50 µl of test solution *b* (250 ppm uracil, dissolved in water) respectively.
- Position of sample vial: *1A1*
- Injection volume: 10 µl, 20 µl, 30 µl, 40 µl, 50 µl
- Repetition: 3

**Note** Fill consecutive positions on the microtiter plates with test solution.

### Analyzing the Individual Chromatograms

1. Calculate the correlation coefficient *r* of the regression lines from the measured values for the *Peak* areas and the injection volume.
2. Enter the results into the *Test Report* form.

### Formula for Determining the Correlation Coefficient

$$r = \frac{\sum x_i y_i - n\bar{x}\bar{y}}{\sqrt{\left(\sum x_i^2 - n\bar{x}^2\right)\left(\sum y_i^2 - n\bar{y}^2\right)}}$$

$y_i$ = *Y* value of measured value *i* (injection volume)

$x_i$ = *X* value of measured value *i* (peak area)

$\bar{y}$ y = arithmetic mean of *Y* across all *n* measured values

$\bar{x}$ x = arithmetic mean of *X* across all *n* measured values

*n* = number of measured value pairs

# 4. Mixture Test

To create a mixing method, follow the instructions in the manual of the chromatography software.

- The test solution variation coefficient *VK2* must not exceed 0.5 %.
- The dilution variation coefficient *VK2* must not exceed ≤ 0.5 %.
- The dilution factor *F10* has to be within the range of 9.85 < x < 10.25.
- Test solution: 10 µl (50 ppm uracil, dissolved in water)
- For injecting the dilution, go to *Mix methods* and create a mixing method in which 40 µl of the test solution is mixed with 360 µl eluent.
  - Dilution: 10 µl, 5 ppm uracil, dissolved in deionized water
- Inject 10 µl test solution and und 10 µl dilution three times each.

### Creating a Sequence with 2 Lines

- Injection volume: 10 µl
- Repeats: 3

## Positioning Vials for Dilution

- To position the vials on the sample plates, select the *Columns* option in the chromatography software.
- Put a vial with test solution (*Sample*) at position *2A1* of the vial plate.
- Put an empty vial (*Destination*) at position *2A5* of the vial plate.
- Put a vial with eluent (*Reagent A*) at position *1A1* of the vial plate.

**Note** Observe the plate assignments for the vials (*Sample*, *Reagent A, Reagent B*, *Destination*).

## Analyzing the Individual Chromatograms

1. Calculate the mean of the measuring values of the *Peak* areas for the test solution and the dilution.
2. Calculate the variation coefficient $VK_2$ .
3. Calculate the variation coefficient $VK_3$ .
4. Calculate the dilution factor *F10* from the ratio of the mean of the *Peak* areas of the test solution and the dilution.
5. Enter the results into the Test Report form.

# Archiving

- Enter all test results into the *Test Report* form.
- Enter the serial number, date of the test, date of the next test and name of the tester.
- File the *Test Report* form in the device logbook.

# Test Report

| Module | Autosampler | |
|---|---|---|
| Autosampler 3950 | Standard ☐ | Sample cooling ☐ |
| Serial number | | |

| No. | Test | Setting | Specification | Result |
|---|---|---|---|---|
| 1 | Reproducibility | ▪ Inject 10 µl test solution nine times.<br>▪ Microtiter plate: Inject 10 µl test solution seven times. | $VK_1 \leq 0.5$ % | |
| 2 | Carryover | Alternately inject 10 µl test solution and 10 µl eluent three times. | $PV \leq 0{,}3$ % | |
| 3 | Linearity | Inject 10, 20, 30, 40 and 50 µl test solution three times each. | $r \geq 0{,}998$ | |
| 4 | Mixture test | Inject 10 µl test solution and 10 µl of the dilution created by the autosampler, three times each. | $VK_2 \leq 0.5$ %<br>$VK_3 \leq 0.5$ %<br>F10: 9,85 < x < 10,25 | |

Date:

Date of the next device test:

Tester:

Signature:

# *Operation Qualification (OQ)* with ChromGate®

**Comprehensive functionality test**

Extensive test of the autosampler functionality using ChromGate® chromatography software.

**Start *OQ***

1. Select*Control⇒Qualification procedures* . The window for selection of a performance qualification opens under *Available Procedures*.
2. In the *Available procedures* window, choose an OQ test for the autosampler and choose *Begin New Qualification Session*.
3. Select *[Start Session]*. The Introduction window opens for the function test.
4. Select *[Continue]* to display the required materials in the chromatography software.
5. Select *[Continue]* to display the settings for the *OQ* test in the chromatography software.

**Select automatic test**

6. Select *Perform all tests automatically*.
7. Select *Active channel* of the detector.
8. Select pump and click [Continue] to apply the settings.

**Start *OQ***

9. Click *[Continue]* to perform the function test. The chromatography software sets the flow rate to 1 ml/min and the wavelength of the detector to 274 nm. You can print out the test results.

**Printing out the results of the function test**

10. Select *[Print report]* to print the function test of the autosampler.

**Quit *OQ***

11. Select *[Exit]* to end the function test of the device.

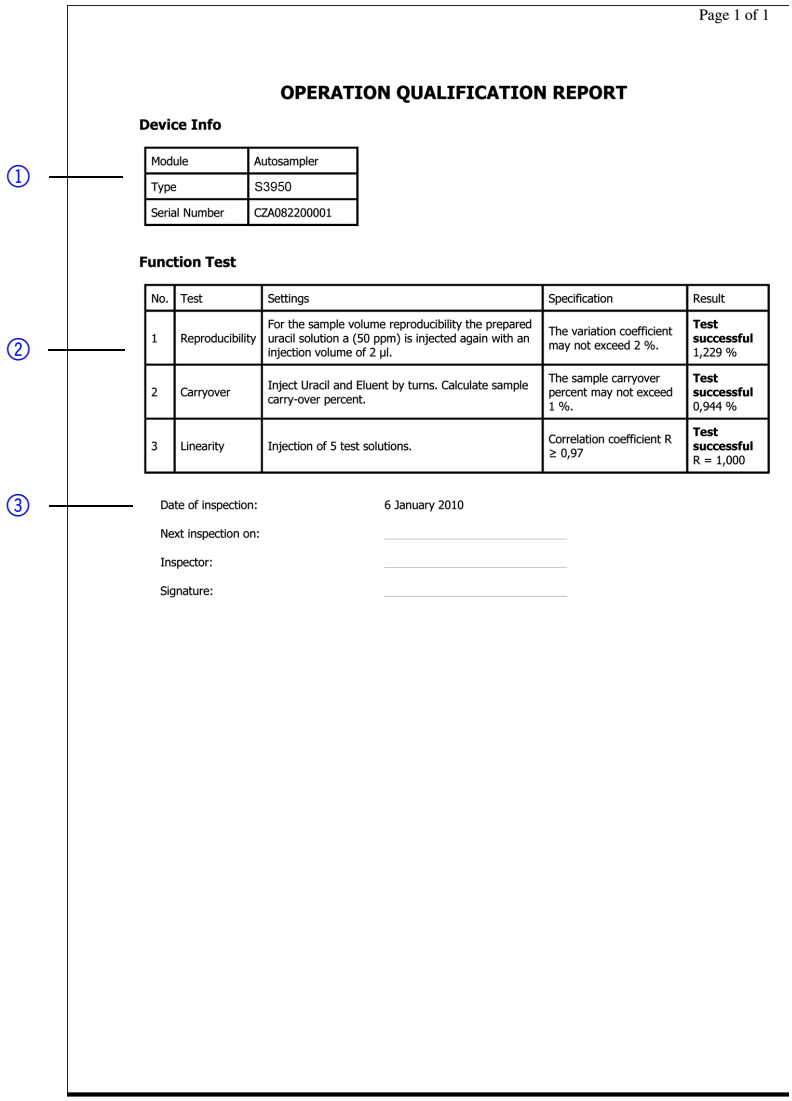
# Operation Qualification Report

## Legend

① Detailed information of the autosampler

② Detailed function test

③ Date and fields for signature and other information



Page 1 of 1

**OPERATION QUALIFICATION REPORT**

**Device Info**

| | |
|---|---|
| Module | Autosampler |
| Type | S3950 |
| Serial Number | CZA082200001 |

**Function Test**

| No. | Test | Settings | Specification | Result |
|---|---|---|---|---|
| 1 | Reproducibility | For the sample volume reproducibility the prepared uracil solution a (50 ppm) is injected again with an injection volume of 2 µl. | The variation coefficient may not exceed 2 %. | **Test successful** 1,229 % |
| 2 | Carryover | Inject Uracil and Eluent by turns. Calculate sample carry-over percent. | The sample carryover percent may not exceed 1 %. | **Test successful** 0,944 % |
| 3 | Linearity | Injection of 5 test solutions. | Correlation coefficient R ≥ 0,97 | **Test successful** R = 1,000 |

Date of inspection: 6 January 2010

Next inspection on: _____

Inspector: _____

Signature: _____



**Fig. 56 Operation Qualification Report**

# Maintenance and Care

## Contacting the Technical Support

You have various options to contact the Technical Support:

**Phone** +49 30 809727-111

**Fax** +49 30 8015010

**E-mail** support@knauer.net

You can make your requests in English and German.

## Maintenance Contract

The following maintenance work on the device may only be performed by KNAUER or a company authorized by KNAUER and is covered by a separate maintenance contract:

- Opening the module
- Removing the hood or the side panels.

## Which Type of Maintenance Tasks May Users Perform on the Device?

Users may perform the following maintenance tasks themselves:

- Exchanging the fuses
- Exchanging the air and sample needle
- Exchanging the injection valve
- Exchanging the sample loop
- Exchanging the rotor seals
- Exchanging the capillary and tubing

**CAUTION! Maintaining a switched on device can cause damage to the device.**
**Switch off the power switch and pull the power plug.**

## Leaks in the Capillary Screw Fittings

**Note** If leaks occur on the capillary screw fittings after maintenance and proper assembly, do not tighten them further, but instead replace them with new connection capillaries.

## Exchanging the Fuses

1. Switch off the autosampler and remove the power plug to completely disconnect the device from the power supply.
2. Remove the fuses from the fuse box at the rear of the device.
3. Insert new fuses (2 x 2.5 A).
4. Plug in the power plug.

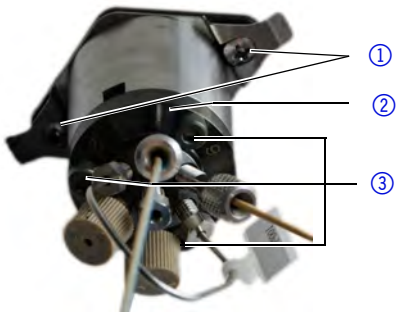
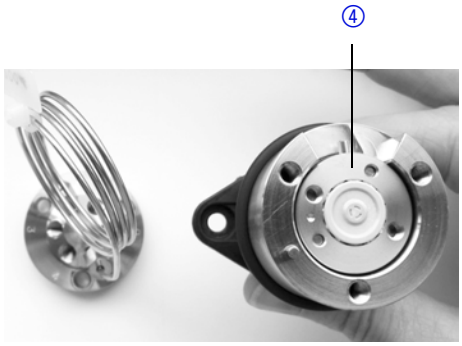
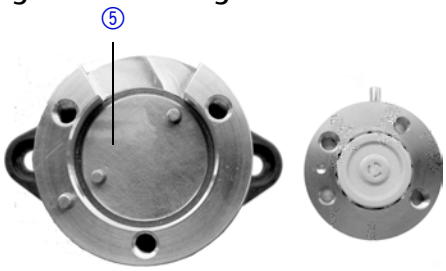
## Changing the Valve and Rotor Seal

- Regularly clean the rotor seal of the injection valve.
- Regularly replace the rotor seal (approx. every three years).
- Remove the front panel of the autosampler.
- Remove the capillary connections, except the sample loop, from the valve.
- During removal, consecutively loosen all screws by half a turn respectively, until they can be removed.

**Note** Do not remove the screw in the cover plate hole diagonal to the valve!

## Removing the Injection Valve and Rotor Seal

**Procedure**

| Process | Figure |
|---|---|
| 1. With a screwdriver, remove screw ① on both sides of the injection valve housing.<br>2. Remove the injection valve.<br>3. With a hexagon wrench key, remove the screws ③ from the valve stator block ② .<br>4. Carefully remove the stator.<br>5. Remove the rotor seal ④ from the rotor ⑤ .<br>6. Clean or exchange the rotor seal. |  <br>**Fig. 57 Disassembling the valve**<br>  <br>**Fig. 58 Removing the rotor seal**<br>  <br>**Fig. 59 Rotor and rotor seal** |

## Installing the Injection Valve

- During installation, hold the injection valve in such a way that the bore hole for connecting the steel capillary to the pump (port 1) is facing upward.
- Alternately tighten all screws by half a turn, until all screws have been fully tightened.

**Legend**

① Labeling, port 1

② Connection from capillary to pump

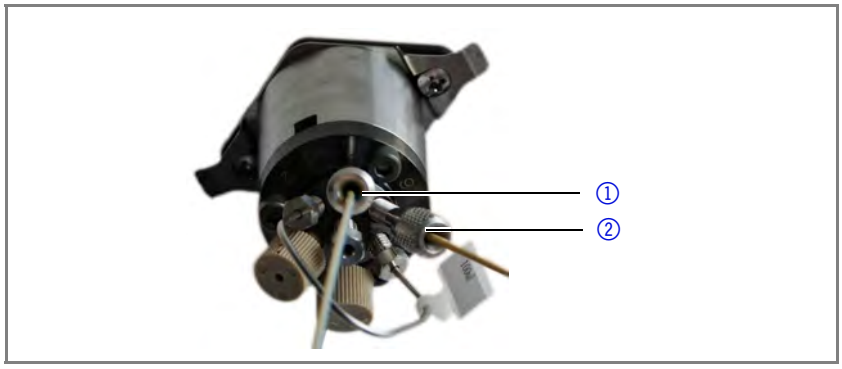



**Fig. 60 Installing the valve**

**Procedure**

1. Insert the rotor seal.
2. Place the valve stator block on the valve rotor and use a hexagon wrench key to tighten the screws.
3. Insert the injection valve and use a screwdriver to tighten the screw on both sides of the valve housing.
4. Connect the capillary again.

# System Flushing

**Procedure**

1. Connect the autosampler to the power supply.
2. Establish a connection to the PC.
3. Start *Autosampler 3950 Service Manager*.
4. Select the *Alias⇒Direct Control* menu.
5. Click *Initialize* to check whether the valve is correctly positioned at the Inject position.
6. In the *Initial Wash* field, click *Start* to flush the system.
7. In the *Initial Wash* field, click *Stop* to stop flushing the system.

# Exchanging the Sample Loop

By standard, the autosampler is equipped with a 100 µl sample loop.

- When assembling a sample loop with a different injection volume, make sure to use the correct combination of syringe and capillaries and configure the controller software appropriately.
- Always connect the sample loop to ports 2 and 5 of the injection valve.
- Calculate the maximum injection volume according to the following formula:
  - Full loop injection:
    Maximum injection volume = loop volume
  - Partial loop injection:
    Maximum injection volume = 50 % loop volume
  - *Microliter pick-up* injection:
    Maximum injection volume = 50 % loop volume - 1.5 x needle volume

# Exchanging the Sample Needle

- When using sample plates with 12 or 48 sample vials, make sure that the needle height setting is >2 mm to prevent the needle from contacting the bottom of the sample vial.
- Tighten the screw fitting until it is finger-tight to prevent the plastic capillary from becoming blocked.

**Legend**

① Fitting

② Plastic capillary

③ Union nut

④ Sample needle

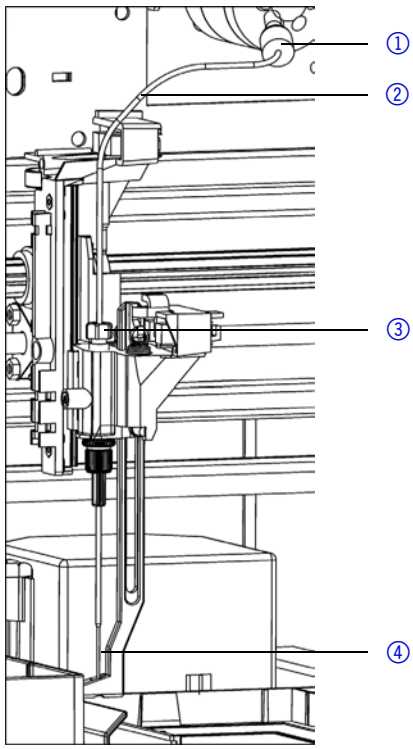



**Fig. 61 Exchanging the sample needle**

**Procedure**

1. Start *Autosampler 3950 Service Manager*.
2. Select the *Alias⇒Direct Control* menu.
3. In the *Needle* field, click *Exchange*. The needle moves to the replacement position.
4. Loosen the union nut ③.
5. Loosen the screw fitting ① of the plastic capillary ② on the injection valve.
6. Remove the sample needle ④ with the plastic capillary.
7. Install a new sample needle unit. Make sure that the air seal fully surrounds the sample needle.
8. Fasten the sample needle with the union nut.
9. Fasten the plastic capillary using the screw fitting on the injection valve.
10. In the *Direct Control* window, click *Initialize*. The needle moves to the initial position.
11. In the *Initial Wash* field, click *Start* to flush the system.
12. In the *Initial Wash* field, click *Stop* to stop flushing the system.
13. Click *Close* to exit the *Direct Control* window.
14. Select the *Alias⇒Adjustments* menu.
15. On the *Needle-Tray* tab, update the settings for the sample plates.

# Exchanging the Air Needle

- When exchanging the air needle, make sure that the thread of the new height adjustment screw is flush with the lower edge of the retaining nut.
- Make sure that the sealing ring is located in the retaining nut.

**Legend**

① Union nut

② Retaining nut

③ Height adjustment screw

④ Air needle

⑤ Sample needle

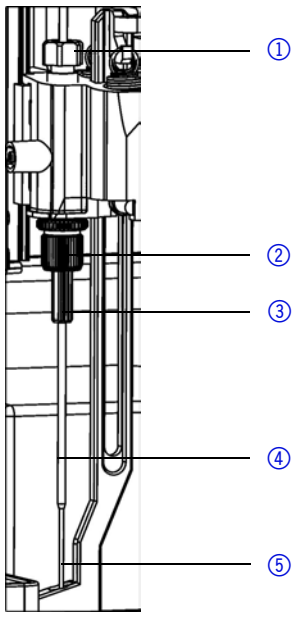



**Fig. 62 Exhanging the air needle**

**Procedure**

1. Start *Autosampler 3950 Service Manager*.
2. Select the *Alias⇒Direct Control* menu.
3. In the *Needle* field, click *Exchange*. The needle moves to the replacement position.
4. Loosen the union nut ①.
5. Loosen the screw fitting of the plastic capillary on the injection valve.
6. Remove the sample needle ⑤ with the plastic capillary.
7. Loosen the retaining nut ② and pull it downwards together with the air needle ④.
8. Unscrew the retaining nut from the height adjustment screw ③.
9. Screw a new air needle with a new height adjustment screw into the retaining nut.
10. Screw in the retaining nut.
11. Insert the sample needle and fasten with the union nut.
12. Fasten the plastic capillary using the screw fitting on the injection valve.
13. In the *Direct Control* window, click *Initialize*. The needle moves to the initial position.
14. In the *Initial Wash* field, click *Start* to flush the system.
15. In the *Initial Wash* field, click *Stop* to stop flushing the system.
16. Click *Close* to exit the *Direct Control* window.
17. Select the *Alias⇒Adjustments* menu.
18. On the *Needle-Tray* tab, update the settings for the sample plates.

# Exchanging the Syringe

By standard, the autosampler is equipped with a 500 µl syringe.

Use isopropanol as flushing solution to remove air bubbles from the new syringe.

**Legend**

① Syringe valve

② Syringe

③ Syringe drive

④ Syringe plunger

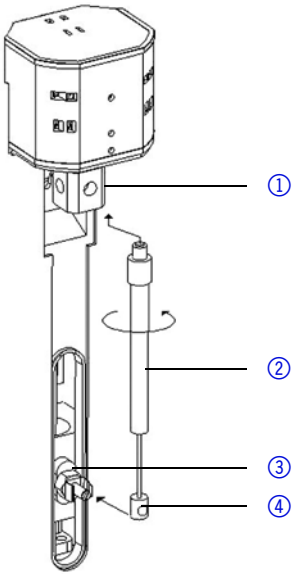



**Fig. 63 Exchanging the syringe**

**Exchanging the Syringe**

1. Start *Autosampler 3950 Service Manager*.
2. Select the *Alias⇒Direct Control* menu.
3. In the *Syringe* field, click *Exchange*. The syringe plunger is lowered.
4. Unscrew the syringe ② by rotating it counterclockwise; leave the adapter in the syringe valve ①.
5. Remove the syringe plunger ④ from the syringe drive ③.
6. Fill new syringe with flushing solution.
7. Insert the syringe plunger into the syringe drive.
8. Tighten the syringe in the syringe valve by rotating it clockwise.
9. In the *Syringe* field, click *Home*. The syringe content is emptied into the drainage tube.
10. If there is still air in the syringe, click *End* in the *Syringe* field. One syringe volume is aspirated into the syringe through the flushing solution tube.
11. In the *Syringe* field, click *Home*. The syringe content is emptied into the drainage tube.
12. Slightly tap the body of the syringe if it still contains air. If necessary, repeat step 10 and 11. Repeat the step.
13. In the *Initial Wash* field, click *Start* to flush the system.
14. In the *Initial Wash* field, click *Stop* to stop flushing the system.
15. Click *Close* to exit the *Direct Control* window.

## Exchanging the Syringe Plunger or Plunger Tip

1. Start *Autosampler 3950 Service Manager*.
2. Select the *Alias⇒Direct Control* menu.
3. In the *Syringe* field, click *Exchange*. The syringe plunger is lowered.
4. Remove the syringe (see above).
5. Pull the syringe plunger out of the glass cylinder of the syringe.
6. Use a pair of tweezers to remove the plunger tip.
7. Wet the new plunger tip with isopropanol.
8. Mount the new plunger tip onto the syringe plunger.
9. Push the syringe plunger into the glass cylinder of the syringe.
10. Install the syringe (see above.)
11. In the *Syringe* field, click *Home*. The syringe content is emptied into the drainage tube.

## Exchanging the Syringe Valve

The syringe valve has four connections, one of them remains unused.

- Hand-tighten all fittings on the connections to the syringe valve.
- To exchange the valve, set it to the Waste position, because the hexagon socket screws are only accessible if the valve is in this position.

**Legend**

① Upper hexagon socket screw

② Lower hexagon socket screw

③ Connection flushing solution tube (hidden)

④ Connection buffer tube

⑤ Connection syringe

⑥ Unused connection

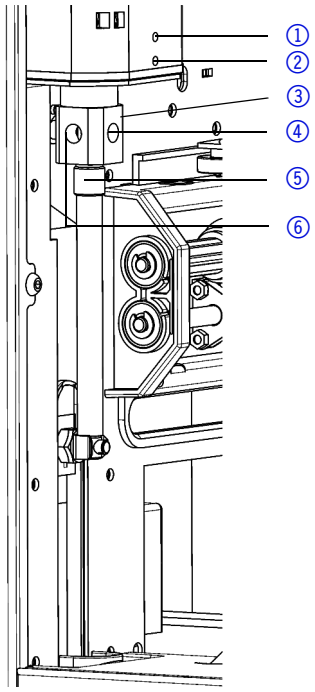



**Fig. 64 Exchanging the syringe valve**

**Procedure**

1. Start *Autosampler 3950 Service Manager*.
2. Select the *Alias⇒Direct Control* menu.
3. In the *Syringe* field, click *Exchange*. The syringe plunger is lowered.
4. Loosen the lower hexagon socket screw by 2 turns.
5. Loosen the upper hexagon socket screw by 2 turns.
6. Pull out the upper part of the syringe.
7. Remove the syringe.
8. Exchange the syringe valve.
9. Insert a new syringe.
10. Tighten the hexagon socket screws.

# Cleaning and Caring for the Device

**CAUTION! Intruding liquids can cause damage to the device!**
**Place solvent bottles next to the device or on a solvent tray.**
**Moisten the cleaning cloth only slightly.**

All smooth surfaces of the device can be cleaned with a mild, commercially available cleaning solution, or with isopropanol.

- Clean collecting container and vial plates with a soft cloth.
- To remove deposits, flush the drainage tube regularly using solvent.

# Disposal

Drop-off old devices at the certified waste facilities, where they will be disposed of properly.

**AVV marking**

According to the German "Abfallverzeichnisverordnung" (AVV) (January, 2001), old devices manufactred by KNAUER are marked as waste electrical and electronic equipment: 160214

**WEEE registration**

KNAUER as a company is registered by the WEEE number DE 34642789 in the German "ElektroAltgeräteRegister" (EAR). It belongs to category 8, under which fall all medical devices and laboratory equipment.

Within the meaning of the WEEE directive, all distributors and importers are responsible for the disposal of old devices. End-users can send their old devices, which must have been manufactured by KNAUER, back to the distributor, the importer, or the company free of charge, but would be charged for their disposal.

# Troubleshooting

## Device Errors

One possible cause of device errors is a malfunctioning valve.

### Checking the Valve

Remove the valve and check all parts for wear and contamination. After the problem has been eliminated and the valve reinstalled, perform the following steps:

**Procedure**

1. Select the *Alias⇒Direct Control* menu.
2. In the *Direct Control* window, click *Initialize*. The needle moves to the initial position.
3. In the *Initial Wash* field, click *Start* to flush the system.
4. In the *Initial Wash* field, click *Stop* to stop flushing the system.
5. Click *Close* to exit the *Direct Control* window.

## Software Faults

Software faults can occur due to flawed communications between the devices or incorrect installation of the software.

**Procedure**

1. Check the cable connections.
2. Start *Autosampler 3950 Service Manager*.
3. Select the *Alias⇒Direct Control* menu.
4. In the *Direct Control* window, click *Initialize*.

## Analytical Errors

Possible causes:

- Wear due to errors in the injection and method settings.
- Unsuitable combination of sample loop, buffer tube and syringe.
- External effects such as temperature, and light-sensitive samples being exposed to light.

Solutions:

- Check whether the application has run previously without errors and that no changes have been made to the analytical system.
- Determine whether the fault is caused by the autosampler or other devices in the system.

If the required degree of reproducibility is not achieved, check the following possible sources of error and instigate steps to eliminate them:

| Cause of fault | Elimination |
|---|---|
| Air in liquid path | Initialize the Autosampler 3950. |
| Leaking syringe | ▪ If the syringe is leaking at the top, check whether it has been installed correctly.<br>▪ If the syringe is leaking at the bottom, exchange the syringe plunger. |
| Leaking syringe valve | Check valve and exchange if required. |
| Rotor seal worn | Exchange the rotor seal and check the stator block of the valve. |
| Dead volume in capillary connections | Install new fittings onto capillary connections. |

If an empty sample run returns an excessively large peak, check the following possible causes of error and instigate steps to eliminate them:

| Cause of fault | Elimination |
|---|---|
| Solubility problems | Either modify sample or accept carry-over. |
| Interaction between the empty sample and the *hardware* | ▪ Check *hardware*:<br>- Flush needle (inside and outside) or install a different needle type (steel, PEEK or with glass coating).<br>- Valve: Exchange rotor seal (other material).<br>- Capillaries and tubing: Use other connections between the autosampler and the columns (steel, PEEK) or other flushing solutions. |
| Empty sample contaminated | Use new empty sample. |
| Cause unknown. | Attempt to solve problem by using different solvents and eluents. |

If no injection is performed:

| Cause of fault | Elimination |
|---|---|
| Liquid path blocked | 1. Disconnect the plastic capillary of the needle from the injection valve.<br>2. Start system flushing.<br>3. If solvent escapes at the injection valve connection to the needle, check the needle.<br>4. If no solvent escapes at the injection valve connection to the needle, disconnect the buffer tube from the injection valve.<br>5. Start system flushing.<br>6. If solvent flows out at the open end of the buffer tube, check the rotor seal.<br>7. If no solvent flows out of the open end of the buffer tube, disconnect the buffer tube from the syringe valve.<br>8. Start system flushing.<br>9. If solvent flows out of syringe valve, check the buffer tube.<br>10. If no solvent flows out of the syringe valve, check whether the connections of the liquid path have been tightened too much. |
| Leaking valve | 1. Unscrew the plastic capillary leading to the needle from the injection valve.<br>2. Disconnect the plastic capillary leading to the syringe from the injection valve.<br>3. Connect the HPLC pump to the injection valve.<br>4. Close the connection to the column at the injection valve.<br>5. Start the pump at a low flow rate.<br>6. Check the connections to the syringe and to the needle at the injection valve to ensure that they are tight.<br>7. If liquid escapes there, check the rotor seal.<br>8. If no liquid escapes there, check the HPLC with a manual valve. |

# System Messages from ChromGate®

The various system messages of the ChromGate® chromatography software from KNAUER are explained below. The system messages are sorted alphabetically.

| System message | Explanation |
|---|---|
| *Autosampler is in run mode.* | ▪ Quit the control software and restart.<br>▪ Switch the device off and on. |
| *Autosampler is not responding. Please check communication settings and ensure the device is online.* | Switch the device off and on. Check the network settings. Inform the Technical Support of the manufacturer in case the system message repeats itself. |
| *Cannot run autosampler.* | Switch the device off and on. Check the network settings. Inform the Technical Support of the manufacturer in case the system message repeats itself. |
| *Cannot set destination vial to %d.* | Check parameters in control software and correct entry. |
| *Cannot set first transport vial to %d.* | Check parameters in control software and correct entry. |
| *Cannot set last transport vial to %d.* | Check parameters in control software and correct entry. |
| *Cannot stop autosampler.* | Check the network settings. Inform the Technical Support of the manufacturer in case the system message repeats itself. |
| *Communication port for autosampler was not initialized. Please check the configuration settings.* | Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself. |
| *Configuration settings do not match with the device. Run cannot start.* | Check configuration and settings. |
| *Destination position not reached.* | Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself. |
| *Deviation of more than +/- 2 mm towards home.* | ▪ Look for visible obstructions in area of vial plate.<br>▪ Check the belt tension of the vial plate. |

| System message | Explanation |
|---|---|
| *Dispenser error.* | Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself. |
| *Electronics error.* | Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself. |
| *EEPROM error in adjustments.* | Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself. |
| *EEPROM error in log counter.* | Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself. |
| *EEPROM error in settings.* | Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself. |
| *EEPROM write error.* | Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself. |
| Error 369 | Not enough transport liquid in store. Refill transport liquid. |
| Error 370 | Not enough reagent in store. Refill reagent. |
| *Error by setting Mix&Dilute vials.* | Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself. |
| *Error occurred during initialization, the Autosampler AS-1 cannot start.* | Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself. |
| *Error resetting output.* | Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself. |
| *Error running user defines program.* | Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself. |

| System message | Explanation |
|---|---|
| *Error setting injection mode.* | Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself. |
| *Error setting needle height.* | Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself. |
| *Error setting injection mode.* | Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself. |
| *Error setting syringe speed.* | Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself. |
| *Error setting the analysis time.* | Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself. |
| *Error setting the auxiliaries.* | Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself. |
| *Error setting the flush time.* | Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself. |
| *Error setting the flush volume.* | Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself. |
| *Error setting the injection volume.* | Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself. |
| *Error setting the loop volume.* | Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself. |
| *Error setting the prep. mode.* | Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself. |

| System message | Explanation |
|---|---|
| *Error setting the syringe volume.* | Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself. |
| *Error setting timed events.* | Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself. |
| *Error setting the tray configuration.* | Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself. |
| *Error setting the tray temperature.* | Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself. |
| *Error setting the vial number.* | Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself. |
| *Error setting tubing volume.* | Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself. |
| *Error setting wash volume.* | Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself. |
| *Flush volume error.* | Check parameters in control software and correct entry. |
| *Home sensor activated when not expected.* | ▪ Check parameters in control software and correct entry.<br>▪ Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself. |
| *Home sensor not de-activated.* | ▪ Check whether there are visible obstructions impairing the vial plate.<br>▪ Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself. |

| System message | Explanation |
|---|---|
| *Home sensor not reached.* | ▪ Check whether there are visible obstructions impairing the vial plate.<br>▪ Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself. |
| *Horizontal: home sensor activated when not expected.* | ▪ Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself. |
| *Horizontal: home sensor not de-activated.* | ▪ Check whether there are visible obstructions impairing the needle unit.<br>▪ Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself. |
| *Horizontal: home sensor not reached.* | ▪ Check whether there are visible obstructions impairing the needle unit.<br>▪ Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself. |
| *Horizontal: needle position is unknown.* | Initialize the needle unit using the control software. |
| *Illegal sensor readout.* | Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself. |
| *Incorrect first destination vial.* | Check parameters in control software and correct entry. |
| *Injection needle unit error.* | ▪ Check whether there are visible obstructions impairing the needle unit.<br>▪ Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself. |
| *Injection valve or ISS unit error.* | Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself. |

| System message | Explanation |
|---|---|
| *Injection volume %.2f is invalid. For specified injection method, volume should be within the range %.2f µl-%.2f µl, with %2f. µl increments.* | Check parameters in control software and correct entry. |
| *Injection volume error.* | Check parameters in control software and correct entry. |
| *Invalid %s vial position %02d. The vial position must be between 01 and %02d.\n.* | Check parameters in control software and correct entry. |
| *Invalid combination of the trays. The combination of different trays for the Mix&Dilute mode is not allowed.* | ▪ Insert the correct vial plate.<br>▪ Check parameters in control software and correct entry. |
| *Invalid combination of the trays. The combination of plates 384 low and 96 high is not allowed.* | ▪ Insert the correct vial plate.<br>▪ Check parameters in control software and correct entry. |
| *Invalid configuration. ISS option not installed on autosampler. Please switch off this option in configuration dialog.* | Check parameters in control software and correct entry. |
| *Invalid configuration. SSV option not installed on autosampler. Please switch off this option in configuration dialog.* | Check parameters in control software and correct entry. |
| *Invalid flush volume %d µl. The flush volume should be between 0 and %d µl.* | Check parameters in control software and correct entry. |
| *Invalid flush volume %2f µl. The flush volume should be between 0 and %2f µl.* | Check parameters in control software and correct entry. |
| *Invalid input. Only values with increments of %.2f allowed.* | Check parameters in control software and correct entry. |
| *Invalid integer number.* | Check parameters in control software and correct entry. |

| System message | Explanation |
|---|---|
| *Invalid instrument is detected.* | Check parameters in control software and correct entry. |
| *Invalid loop volume %d µl. The loop volume should be between 0 and %d µl.* | Check parameters in control software and correct entry. |
| *Invalid loop volume %2f µl. The loop volume should be between 0 and %2f µl.* | Check parameters in control software and correct entry. |
| *Invalid mix program: no Destination vial is specified in the configuration dialog.* | Check parameters in control software and correct entry. |
| *Invalid mix program: no Reagent A vial is specified in the configuration dialog.* | Check parameters in control software and correct entry. |
| *Invalid mix program: no Reagent B vial is specified in the configuration dialog.* | Check parameters in control software and correct entry. |
| *Invalid mix times. The time should be between 1 and 9.* | Check parameters in control software and correct entry. |
| *Invalid needle height %d mm. The needle height should be between %d and %d mm.* | Check parameters in control software and correct entry. |
| *Invalid time-based method. Several AUX events have the same time.* | Check parameters in control software and correct entry. |
| *Invalid time-based method. Several SSV events have the same time.* | Check parameters in control software and correct entry. |
| *Invalid tray temperature %d ˚C. The temperature should be between 4 and 22 ˚C.* | Check parameters in control software and correct entry. |
| *Invalid tray configuration: two or more vial positions are the same.* | Check parameters in control software and correct entry. |

| System message | Explanation |
|---|---|
| *Invalid tubing volume %d µl. The tubing volume should be between 0 and %d µl.* | Check parameters in control software and correct entry. |
| *Invalid tubing volume %2f µl. The tubing volume should be between %2f and %2f µl.* | Check parameters in control software and correct entry. |
| *Invalid wait time. The time should be between 0 and 9 h 50 min 59 sec.Invalis wash volume %d µl. The wash volume should be between %d and %d µl* | Check parameters in control software and correct entry. |
| *Invalid volume %d µl. The volume should be between the 0 and the syringe volume (%d µl).* | Check parameters in control software and correct entry. |
| *ISS valve error.* | Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself. |
| *Missing destination vial.* | ▪ Check position of sample vial.<br>▪ Check parameters in control software and correct entry. |
| *Missing reagent vial.* | ▪ Check position of sample vial.<br>▪ Check parameters in control software and correct entry. |
| *Missing transport vial.* | ▪ Check position of sample vial.<br>▪ Check parameters in control software and correct entry. |
| *Needle movement error.* | ▪ Check position of needle unit.<br>▪ Switch the device off and on. |
| *Missing vial.* | ▪ Check position of needle unit.<br>▪ Switch the device off and on. |
| *Missing wash vial error.* | ▪ Check position of needle unit.<br>▪ Switch the device off and on. |
| *No destination vial is specified in the configuration.* | Check parameters in control software and correct entry. |
| *No reagent A vial is specified in the configuration.* | Check parameters in control software and correct entry. |

| System message | Explanation |
|---|---|
| *No reagent B vial is specified in the configuration.* | Check parameters in control software and correct entry. |
| *No transport vials are defined in the tray configuration. It is not possible to use the µl pick-up injection mode.* | Check parameters in control software and correct entry. |
| *No user defined or mix program is running.* | Check parameters in control software and correct entry. |
| *Not enough reagent liquid.* | Check volume of liquid and change as required. |
| *Not enough transport liquid available due to missing transport vials.* | Check volume of liquid and change as required. |
| *Please specify inject marker or AUX event to be able to trigger the run.* | Check parameters in control software and correct entry. |
| *Selecting transport position failed.* | Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself. |
| *Serial number is not valid. Please check the configuration.* | Check parameters in control software and correct entry. |
| *Setting mix program error.* | Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself. |
| *Setting service mode failed.* | Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself. |
| *Syringe dispenser unit error.* | Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself. |
| *Syringe home sensor not de-activated.* | ▪ Needle flushing with control software.<br>▪ Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself. |

| System message | Explanation |
|---|---|
| *Syringe home sensor not reached.* | ▪ Needle flushing with control software.<br>▪ Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself. |
| *Syringe position is unknown.* | Initialize the syringe unit using the control software. |
| *Syringe rotation error.* | ▪ Needle flushing with control software.<br>▪ Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself. |
| *Syringe valve did not find destination position.* | ▪ Needle flushing with control software.<br>▪ Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself. |
| *Temperature above 48 °C at cooling ON.* | ▪ Switch off the cooling and check whether ambient temperature sensor is properly functioning.<br>▪ Inform the Technical Support of the manufacturer in case the system message repeats itself. |
| *The 10 ml syringe cannot be used for standard injections.* | Exchange the syringe. |
| *The autosampler has detected another tray than that which is currently configured. Please select the correct tray in configuration dialog.* | Check control software configuration and correct entry. |
| *ISS-A option not installed on autosampler. Please switch off ISS-A option in configuration dialog.* | Check control software configuration and correct entry. |
| *ISS-B option not installed on autosampler. Please switch off ISS-B option in configuration dialog.* | Check control software configuration and correct entry. |

| System message | Explanation |
|---|---|
| *Oven option not installed on autosampler. Please switch off oven option in configuration dialog.* | Check control software configuration and correct entry. |
| *The autosampler is not ready. Please try later.* | Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself. |
| *The injection volume of %2f µl is invalid. For the specified injection method, volume should equal %2f µl.* | Check parameters in control software and correct entry. |
| *The sample needle is not in the home position while the tray is rotating.* | Check parameters in control software and correct entry. |
| *Trace from tray cooling cannot be acquired. Tray cooling is off.* | Check parameters in control software and correct entry. |
| *Tray advance is not available at this time.* | Check parameters in control software and correct entry. |
| *Tray error.* | Check parameters in control software and correct entry. |
| *Valve error.* | Check parameters in control software and correct entry. |
| *Vertical: home sensor not de-activated.* | ▪ Check whether there are visible obstructions impairing the needle unit.<br>▪ Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself. |
| *Vertical: home sensor not reached.* | ▪ Check whether there are visible obstructions impairing the needle unit.<br>▪ Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself. |
| *Vertical: needle position is unknown.* | Initialize the instrument in the control software. |

| System message | Explanation |
|---|---|
| *Vertical: stripper did not detect plate (or wash/ waste). Missing vial.* | ▪ Check sample vial and plate.<br>▪ Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself. |
| *Vertical: stripper stuck.* | Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself. |
| *Vertical: The sample needle arm is at an invalid position.* | Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself. |
| *Vial number error.* | Check parameters in control software and correct entry. |
| *Wear-out limit reached.* | Switch the device off and on. If the system message appears again, notify KNAUER Technical Support. The valve must be replaced. |
| *Wrong loop volume. The largest loop volume for standard injections is 1000 µl.* | Check parameters in control software and correct entry. |
| *Wrong tubing volume. The largest tubing volume for standard injections is 200 µl.* | Check parameters in control software and correct entry. |

# Technical Data

**Ambient conditions**

| | |
|---|---|
| Temperature range | 4 – 40 ˚C; 39 – 104 ˚F |
| Air humidity | Below 90 % humidity (non-condensing) |

## Standard Version

**Autosampler 3950**

| | |
|---|---|
| Injection pressure | Up to 700 bar |
| Sample capacity | Microtiter plates for max. 768 wells or sample plates for max. 96 vials |
| Injection volume | 1 – 5000 µl |
| Sample loop | 100 µl |
| Syringe | 500 µl |
| Injection time | <60 s, incl. cleaning |
| Injection modes | PASA™ loop injection principle:<br>▪ Full loop injection<br>▪ Partial loop injection<br>▪ *Microliter pick-up* injection |
| Reproducibility | RSD (Relative Standard Deviation):<br>▪ Full loop injection < 0.3 %<br>▪ Partial loop fill with injection volumes > 10 µl: < 0.5 %<br>▪ Microliter pickup injections with injection volumes > 10 µl: < 1.0 % |
| Carryover | < 0.05 % with needle cleaning |
| Temperature control | 4 – 40 °C[1] |
| Weight | ▪ 19 kg<br>▪ With temperature control: 21 kg |
| Dimensions (length × width × height) | ▪ 510 × 300 × 360 mm<br>▪ With cooling: 575 × 300 × 360 mm |
| Supply voltage range | 100 – 240 V |
| Supply frequency | 50 – 60 Hz |

1) Relevant to Autosampler 3950 with temperature control

# Accessories and Spare Parts

## Device and Accessories

| Name | Order number |
|---|---|
| Autosampler 3950, LAN, Standard | A50060 |
| Autosampler 3950, LAN, cool/heat | A500601 |
| User manual | V1511 |

## Repeat Orders

| Name | Order number |
|---|---|
| Accessory kit for autosampler with vials, caps, septum and pliers for opening and closing | A0664 |
| Vial plates for 1.5 ml vials<br>2 pieces | A50050 |
| 96 well plate, U sanitized, 0.35 ml | A1823 |
| 96 well plate, U sanitized, 1.2 ml | A1823V1 |
| PTFE tubing, 3.2 mm (1/8") OD, 1.5 mm ID, 300 cm length | A0732 |
| Wash bottle 250 ml rectangular | M2054 |
| Syringe 500 µl | M2070 |
| Air needle, white, 62 mm | A50058 |
| Set of air needles | A50059 |
| Air needle, yellow, 50 mm | M20401 |
| Air needle, red, 56 mm | M20402 |
| Air needle, blue, 68 mm | M20403 |
| Air needle, green, 74 mm | M20404 |
| Air needle, black, 80 mm | M20405 |
| 2 × 2.5 A fuse | M2067 |
| Network cable | A5255 |
| RS-232 cable for connection to a PC | A0895 |
| User manual, German | V1511 |
| User manual, English | V1511A |

# Accessories 84 +3

| Name | Order number |
|---|---|
| Vial plate for 84x1.5ml and 3x10ml vials | A500501 |
| 125 Vials 10 ml, 500 crimp caps and 500 septa, ø 22 mm | A1662 |

# Legal Information

## Warranty Conditions

The factory warranty for the device is valid for 12 months after the date of dispatch. All warranty claims shall expire in the event that any unauthorized changes are made to the device.

During the warranty period, any components with material or design-related defects will be replaced or repaired by the manufacturer free of charge.

This warranty excludes the following:

1. Accidental or willful damage
2. Damage or errors caused by third parties that are not contractually related to the manufacturer at the time the damage occurs
3. Wear parts, fuses, glass parts, columns, light sources, cuvettes and other optical components
4. Damage caused by negligence or improper operation of the device and damage caused by clogged capillaries
5. Packaging and transport damage

In the event of device malfunctions, directly contact the manufacturer.

## Manufacturer

Wissenschaftliche Gerätebau
Dr. Ing. Herbert KNAUER GmbH
Hegauer Weg 38
14163 Berlin, Germany
Phone: +49 30 809727-0
Fax: +49 30 8015010
E-Mail: info@knauer.net
Internet: www.knauer.net

## Transportation Damages

The packaging of our devices provides the best possible protection against transportation damage. Check the devices for signs of transportation damages. In case you notice any damage, contact the technical support and the forwarder company within three workdays.

# Declaration of Conformity

**Manufacturer name and address**

Wissenschaftliche Gerätebau
Dr. Ing. Herbert KNAUER GmbH
Hegauer Weg 38
14163 Berlin, Germany

**Autosampler 3950**

Order numbers: A5005V2; A50051V2; A50052V2; A50053V2; A50054V2

The device complies with the following requirements and product specifications:

- DIRECTIVE 2006/42/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 17 May 2006 on machinery, and amending Directive 95/16/EC (recast)
- DIN EN 60799 (June 1999) Electrical accessories - Cord sets and interconnection cord sets
- IEC 61010-1 (2010 + Corrigendum: 2011) Safety requirements for electrical equipment for measurement, control and laboratory use
  - Low voltage directive (2006/95/EC)
- EN 61000-3-2 (2005 + A1:2008 + A2:2009) Electromagnetic compatibility (EMC) Part 3-2
  - EMC standard (2004/108/EC)
- IEC 61326-1 (2006) Electrical equipment for measurement, control and laboratory use – EMC requirements
  - EN 61326-1 Corrigendum 2 (2011)
- Directives for an environmentally sound use of electrical and electronic equipment
  - RoHS directives 2002/95/EC (2003) and 2011/65/EU on the restriction of the use of certain hazardous substances in electrical and electronic equipment
  - WEEE directive 2002/96/EC (2003) and 2012/19/EU on waste electrical and electronic equipment

The product was tested with a typical configuration.

Berlin, 6/3/2013

Dr. Alexander Bünz (Managing Director)

The mark of conformity has been applied to the rear panel of the device.

CE

# Abbreviations and Terminology

Here you can find information on the abbreviations and terminology used in this manual.

| Term | Explanations |
|---|---|
| GLP | Good Laboratory Practice - quality assurance for laboratories. |
| HPLC | High Pressure Liquid Chromatography. |
| HPG | High Pressure Gradient. Operating mode for UHPLC or HPLC systems The solvent is mixed on the high pressure side of the pump. |
| IP address | Unique address of transmitter or receiver in local network or Internet (Internet protocol) |
| solvents | Mobile phase (eluent) or carrier for liquid chromatography. |
| LPG | Low Pressure Gradient. Operating mode for UHPLC or HPLC systems The solvent is mixed on the low pressure side of the pump. |
| Remote | The device is completely controlled by the chromatography software. |
| UHPLC | Liquid chromatography in the ultra-high pressure or ultra-high performance range. |
| Vials | Small bottles used in the sample plates of the autosampler. There are two differents kinds of vials: sample vials and destination vials. |
| Wells | Deepening in microtiter plates, which are being used in the autosampler. |

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